Anticoagulant effects of statins and their clinical implications

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Summary

There is evidence indicating that statins (3-hydroxy-methylglutaryl coenzyme A reductase inhibitors) may produce several cholesterol-independent antithrombotic effects. In this review, we provide an update on the current understanding of the interactions between statins and blood coagulation and their potential relevance to the prevention of venous thromboembolism (VTE). Anticoagulant properties of statins reported in experimental and clinical studies involve decreased tissue factor expression resulting in reduced thrombin generation and attenuation of pro-coagulant reactions catalysed by thrombin, such as fibrinogen cleavage, factor V and factor XIII activation, as well as enhanced endothelial thrombomodulin expression, resulting in increased protein C activation and factor Va inactivation. Observational studies and one randomized trial have shown reduced VTE risk in subjects receiving statins, although their findings still generate much controversy and suggest that the most potent statin rosuvastatin exerts the largest effect.

Keywords

Blood coagulation, statins, tissue factor, thrombin, venous thromboembolism

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Introduction

Statins (3-hydroxy-methylglutaryl coenzyme A [HMG-CoA] reductase inhibitors), both lipophilic agents – atorvastatin, simvastatin and fluvastatin, and hydrophilic agents – rosuvastatin and pravastatin, are effective in reducing low-density lipoprotein (LDL) cholesterol. At typical daily doses statins result in reduction in total and LDL cholesterol by 18–60% with decreases in triglycerides by 10–30% and slight increases in high-density lipoprotein (HDL) cholesterol (8%). Data from the Cholesterol Treatment Trialists' Collaboration found that statins reduced the risk of major vascular events by 22% (1). Compelling clinical evidence indicates that statins also decrease cardiovascular and all-cause death (2).

A number of 'pleiotropic' effects of statins have been suggested to contribute to their efficacy in cardiovascular disease (3). The first clinical data suggesting that statins have benefits beyond lipid lowering came from the Heart Protection Study, in which simvastatin reduced mortality and morbidity even in patients with 'normal' LDL-cholesterol levels (<2.6 mmol/l or 100 mg/dl) (4). Ancillary effects of statins included 1) improvement in endothelial function via increased nitric oxide (NO) bioavailability, 2) suppression of inflammation via reduction in proinflammatory cytokines, chaemokines and adhesion molecule expression, lowering C-reactive protein (CRP), 3) stabilisation of atherosclerotic plaques including modification of the physiochemical properties of the lipid core, 4) reducing smooth muscle cell proliferation, 5) immunomodulation, and 6) inhibition of cardiac hypertrophy (5, 6).

Most of these additional statin-mediated actions reported are independent of blood cholesterol reduction. Statins reduce formation of isoprenoids, altering protein prenylation, a critical event in the posttranslational modification of proteins involved in the regulation of cell cycle progression, proliferation and signalling pathways (7, 8). Inhibition of mevalonic acid synthesis blocks the synthesis of isoprenoid intermediates of the cholesterol biosynthetic pathway (e.g. farnesyl pyrophosphate and geranylgeranylpyrophosphate [GGPP]). These compounds permit the attachment of signalling proteins for the posttranslational modification of GTP-binding protein Ras and Ras-like proteins to the cell membrane. The inhibition of Rho, Ras, and Rac, whose localisation and functions are dependent on isoprenylation, is perceived to be a key process in mediating the statins pleiotropic effects (8). Statins also reduce the stability of lipid raft formation and modulate immune activation and regulation (6).

Antithrombotic properties of statins were recently reviewed by Violi et al. (9) and previously by us (10). In this review, we focus on the effect of statins on blood coagulation and their potential relevance for the prevention of venous thromboembolism (VTE).

Tissue factor (TF)

TF is the major initiator of blood clotting *in vivo*. The formation of the Ca^{2+}-dependent complex between TF and plasma factor (F)VIIa on the cell surfaces leads to the activation of FX and FIX, and subsequent thrombin generation and fibrin formation (▶Fig-

ure 1) (11, 12). In healthy individuals, TF is associated with extravascular tissue and expressed in monocytes. TF expression within atherosclerotic plaques and an increased number of TF-positive microparticles promote intravascular thrombosis (11, 12).

Colli et al. (13) demonstrated that simvastatin and fluvastatin, decreased TF mRNA expression and activity in cultured human monocytes/macrophages stimulated by lipopolysaccharides (LPS). This effect was reversed by co-incubation with mevalonate or GGPP, but not by cholesterol. In contrast, pravastatin at concentrations 100 times greater than effective for lipophilic statins, did not affect TF mRNA expression or activity on monocytes (13). Inhibition of transcription nuclear factor κB (NF-κB) activation has been reported as the mechanism underlying down-regulation of TF expression (13). Down-regulation of TF expression has also been observed on endothelial and vascular smooth muscle cells. Further studies reported that cerivastatin (paper withdrawn, 2001), atorvastatin, simvastatin, pravastatin, lovastatin, and fluvastatin, in this order, suppress NF-κB activation in human monocytes stimulated by LPS from 45% inhibition for cerivastatin to 5% for fluvastatin (14). A three-fold reduction in TF expression and activity was observed in human LPS-stimulated monocytes isolated from hypercholesterolaemic subjects treated with 20 mg/day simvastatin (15).

Simvastatin has been shown to inhibit thrombin-induced TF expression in human aortic endothelial cells and aortic smooth muscle cells (16) while mevalonate reversed this effect. The inhibition of Rho-kinase-dependent Akt dephosphorylation mediates, at least in part, the inhibitory effect of statins on TF expression in the endothelium (16, 17).

Activation of protease activated receptors (PAR) by thrombin induces TF expression in endothelial cells, which requires caveolin-1 membrane microdomains. Banfi et al. (18) have shown that fluvastatin and rosuvastatin block this mechanism of induction of TF expression by inhibition of ERK1/2 phosphorylation, and PAR1 relocation. This statin-induced effect is independent of isoprenoids and may be mediated by decreasing the cholesterol content of the cell membrane (18). Statin-induced suppression of the decryption of TF remains controversial (19, 20).

Down-regulation of TF associated with suppressed inflammation, but not with cholesterol levels, has been documented for various statins in animal models, such as apolipoprotein E-deficient mice and in cholesterol-fed rabbits (21–24). A substantial reduction in vascular TF expression, up to 70%, was observed for rosuvastatin (22). In patients, 4–6 months of treatment with atorvastatin resulted in 29% lower TF antigen levels and 56% lower TF activity in atherosclerotic plaques removed during endarterectomy compared with the values for subjects receiving placebo (25). Statin-induced decrease in TF expression has also been reported in atherosclerotic plaques removed from coronary arteries (26).

The effect of simvastatin on coagulation in LDL receptor-deficient mice on a four-week high-fat diet plus simvastatin decreased oxidised LDL (oxLDL), white blood cell TF expression and the number of TF^+ microparticles. Administration of simvastatin also reduced TF-containing microparticles and circulating thrombin generation markers despite unaltered blood cholesterol levels. The simvastatin-induced changes were associated with prolonged time from arterial injury to the vessel closure. TF expression in hypercholesterolaemic mice and monkeys was mediated by the monocyte CD36/TLR4/TLR6 heterotrimeric complex enhanced by oxLDL and simvastatin reduced expression of TF and interleukin-6 through lowering oxLDL in these animal models (27). It re-

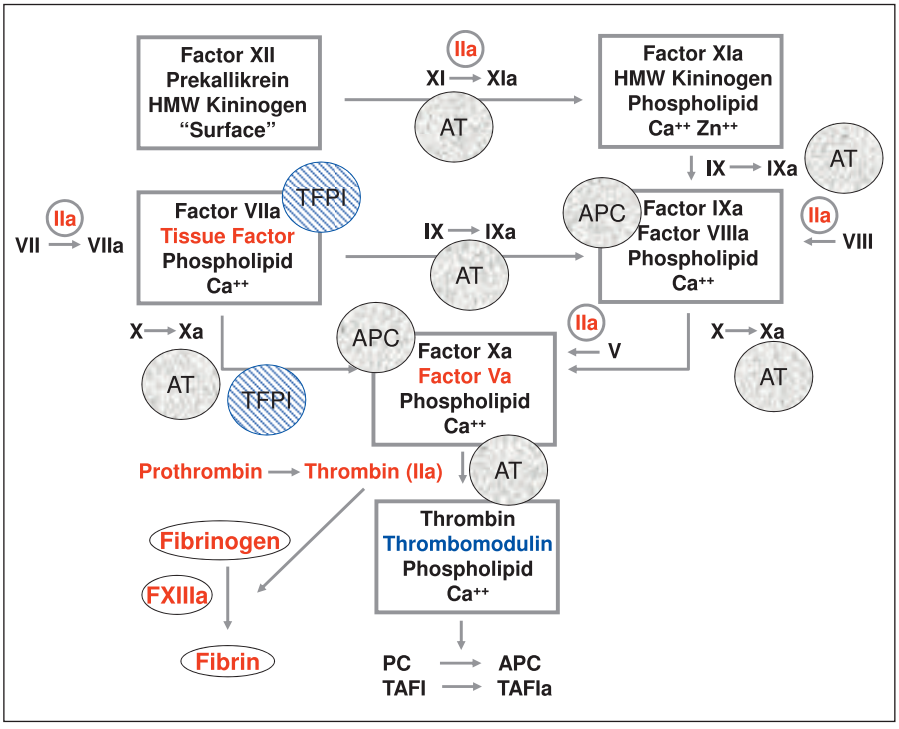

Figure 1: The blood coagulation cascade and proteins or reactions reportedly affected by statin therapy. The inventory and connectivity of the vitamin K dependent complexes (squares; intrinsic tenase, extrinsic tenase, prothrombinase and protein Case) leading to the generation of thrombin (IIa) and the formation of a fibrin clot are illustrated. The square in each of the complexes is representative of an anionic-phospholipid like membrane and is required for assembly and expression of activity. The blood coagulation cascade is down regulated by the inhibitors (hatched circles) antithrombin (AT), tissue factor pathway inhibitor (TFPI) and activated protein C (APC). The proteins or pathways that have been shown to be affected by statins are tissue factor expression, thrombin generation, FV activation, fibrinogen cleavage, FXIII activation, thrombomodulin expression, inactivation of FVa, and TFPI production/activity. Increased effect by statins is shown in red and decreased effect by statins is shown in blue.

mains to be established to what extent those findings are relevant in humans.

Evidence for statin-induced down-regulation of TF expression comes also from cancer studies. Prostate cancer cells release TF-bearing, cholesterol-rich, pro-coagulant, submicron secretory granules surrounded by a membrane, called prostasomes (28). It has been shown that in prostate cancer cells simvastatin led to reduction of TF combined with induction of apoptosis.

Thrombin

Thrombin generation is key to haemostatic plug or pathological thrombosis (29). Thrombin exerts a multitude of functions (►Figure 1), including platelet activation, the clotting of fibrinogen, activation of the crosslinking transglutaminase FXIII, FV activation, enhanced TF expression, endothelial cell activation, stimulation of cell proliferation, and vascular constriction, among others. Thrombin is inactivated by antithrombin with production down-regulated by the dynamic protein C (PC) system.

Experimental and clinical data indicate that atorvastatin, simvastatin, or pravastatin can decrease thrombin formation (►Figure 1). However, measurements of thrombin markers, mostly prothrombin fragments 1.2 (F1.2) or thrombin-antithrombin complexes (TAT), in plasma of venous blood obtained from statin-treated subjects yielded inconsistent results ranging from 20% reduction to a neutral effect (30–32). Most experimental platelet- or monocyte-based thrombin generation models showed dampening of this process in response to various statins in hypercholesterolaemic subjects, with much less convincing evidence for healthy individuals. In recalcified plasma, pravastatin has been shown to decrease platelet-dependent thrombin generation in hypercholesterolaemia (33). Ferro et al. (34) reported a marked inhibition of thrombin generation in plasma incubated with isolated peripheral mononuclear cells obtained from healthy individuals and hypercholesterolaemic subjects subjected to the effect of simvastatin. Although baseline F1.2 formation was higher in hypercholesterolaemic patients, the simvastatin-induced reduction in thrombin generation was similar in both groups regardless of blood cholesterol. This reduction varied from <10% at a concentration of 0.01 μmol/l to 55% at a concentration of 10 μmol/l (34). However, Lindhout et al. (35) failed to show the inhibition of phosphatidylserine-dependent prothrombinase activity by simvastatin 40 mg/day assessed in platelets isolated from healthy individuals treated for two weeks. In another model it was reported that in subjects with elevated cholesterol levels and high cardiovascular risk, simvastatin 40 mg/day may reduce maximum prothrombinase levels and maximum rates of its formation, thus attenuating the prothrombotic consequences of aspirin resistance (36). The methodologies used and patient characteristics may partially explain these discrepancies.

We used a model of microvascular injury (37, 38) with determination of various analytes in consecutive blood samples collected from standardised bleeding-time wounds. This approach combines the effects of blood, the vascular wall, and extravascular tissue to provide data evaluating *in vivo* haemostasis. In patients with total cholesterol above 6.5 mmol/l, a three-month simvastatin treatment (20–40 mg/day) reduced the maximum rate and level of thrombin generation at the site of microvascular injury (39). Patients with coronary artery disease (CAD) and total cholesterol between 5.2 and 6.5 mmol/l had reduced thrombin formation after simvastatin administration (40). In hypercholesterolaemic subjects, a three-month administration of 20-40 mg/day simvastatin was associated with 16% reduction in the rate of prothrombin depletion and 27% decrease in the rate of formation of prothrombin activation products (41). In these patients, a longer duration of the initiation phase of blood coagulation, the "lag phase," which is largely determined by the activity of the TF-FVIIa complex (extrinsic tenase complex) was observed (41). These anticoagulant effects of simvastatin showed no association with the pre- or post-treatment blood cholesterol concentrations (41).

Importantly, in patients with a high risk of CAD and with LDL cholesterol >3.4 mmol/l, as few as three and 28 days of 40 mg/day simvastatin lowered plasma TAT and total thrombin generated at the site of microvascular injury by 30%; the effects were independent of cholesterol reduction (42). Similar effects were observed after one month of atorvastatin administration (40 mg/day), with rates of formation of thrombin formation decreased by 34–40% (43). Sanguigni et al. (44) reported in hypercholesterolaemic patients that atorvastatin 10 mg/day given for three days decreases plasma F1.2 suggesting an early anticoagulant effect, which was probably associated with statin-induced down-regulation of CD40 ligand (CD40L) that enhances TF expression and thrombin generation. Similarly, simvastatin (40 mg/day for three days) in hypercholesterolaemia resulted in significant reduction in the rate of thrombin generation triggered by vascular injury (45). Evidence suggests that three doses of most statins are sufficient to reduce thrombin formation.

In acute coronary syndromes (ACS) in which enhanced blood thrombogenicity is triggered by the rupture of a coronary atherosclerotic plaque (46) studies failed to show reduced thrombin generation in circulating blood even at high-dose atorvastatin (47). Conversely, it has been observed that statin use (one month or longer) is associated with lower thrombin generation and platelet activation at the site of vascular injury determined within the first 12 hours of acute myocardial infarction, but not cholesterol levels (48). These results imply that while statins may be too weak to overcome potent prothrombotic mechanisms, they may favourably modulate thrombogenicity in ACS.

Several studies have shown that statins are effective in lowering thrombin generation in type 2 diabetes which is commonly associated with dyslipidemia, hypercoagulable state and enhanced inflammation (49). A significant reduction in thrombin generation assessed by a calibrated automated thrombogram was also observed in diabetic patients with no previous cardiovascular events who received atorvastatin (40 mg/day) (50). Pravastatin given for eight weeks to diabetic patients was also shown to reduce thrombin generation markers together with lower CRP and unaltered fibrinogen (51). Reduced thrombin generation markers in plasma were observed also in elderly patients with atrial fibrillation on

oral anticoagulation (52), those with peripheral artery disease (53) and after percutaneous coronary intervention (54).

The magnitude of statin-induced reduction of thrombin generation is in part genetically determined. A genetic polymorphism reported to affect statin action is the +5466G allelic variant of the TF gene, which occurs in 16% of Europeans. It has been demonstrated that in carriers of the +5466G allele at high cardiovascular risk there was a greater decrease in the rate of thrombin formation following three-month simvastatin administration compared with those with the +5466AA genotype (55).

Of note, Dietzen et al. (56) suggested that that the effect of statins on membrane content contributes to their anticoagulant properties. In EA.hy926 cells and normal adult human fibroblasts, atorvastatin at low concentration (1 μmol/l) limits exposure of PS by restricting the cellular pool of isoprenoids (56).

Taken together, statins can inhibit coagulation via largely cholesterol-independent mechanisms. However, the largest reductions in thrombin formation have been observed in hypercholesterolaemia. These actions of statins are most pronounced under conditions triggered by vascular injury, or *in vitro* in the presence of monocytes. Despite some inconsistencies, most models are highly suggestive of reduced thrombin formation in response to various statins, with the strongest evidence for simvastatin, pravastatin and atorvastatin.

Fibrinogen and fibrin

Thrombin catalyses the release of fibrinopeptides from fibrinogen to form fibrin monomers which polymerise (►Figure 1). The observed effects of statins on fibrinogen concentrations include their increase, decrease, or most often, no significant change (51, 57–62). Recent data from the Multi-Ethnic Study of Atherosclerosis (MESA) study of healthy men and women, free of clinical cardiovascular disease at baseline, showed that statin users had slightly higher fibrinogen (2%) and plasminogen activator inhibitor-1, PAI-1 (22%) levels as compared with non-users (57). Most data indicates that the changes in fibrinogen concentrations following use of statins are negligible.

Most statins except pravastatin have demonstrated downregulation of the expression of PAI-1 and increase the expression of tissue-type PA (t-PA) in monocytes, smooth muscle cells and endothelial cells (63–65), although atorvastatin upregulates PAI-1 synthesis during the early stages of monocyte/macrophage differentiation, but has no effect on PAI-1 in mature human macrophages (63). Those changes may alter the local fibrinolytic balance within the vessel wall toward increased fibrinolytic capacity. Downregulation of statins on PAI-1 expression is dependent on the inhibition of Rho family proteins and may involve an activation of PI-3 kinase/Akt signalling pathways (66). Given a key role of PAI-1 in fibrinolysis and its binding to the gamma A/gamma' variant of fibrin(ogen) via vitronectin (67), localisation of plasma PAI-1-vitronectin complexes on the surface of fibrin clots may modulate fibrinolysis and clot reorganisation.

Fibrinogen cleavage, as evidenced by increased fibrinopeptide levels, has been shown to be impaired by statins, consistent with reduced thrombin generation. Decreased amounts of fibrinopeptides have been reported in hypercholesterolaemic subjects after a three-month treatment with simvastatin, independent of cholesterol reduction (39, 41). Rates of fibrinogen cleavage were reduced by 32% after 28 days of treatment with atorvastatin 40 mg/day in CAD patients (43).

Statins have also been reported to alter fibrin clot characteristics, in a process partially associated with reduced thrombin formation. A four-week treatment with 40 mg/day simvastatin or atorvastatin resulted in increased fibrin clot permeability by 20%, combined with faster clot lysis in subjects at high cardiovascular risk (68).

Statins can also modulate fibrin clot phenotype in young or middle-aged patients with prior VTE and low cardiovascular risk. In patients after deep-vein thrombosis (DVT), administration of 40 mg/day atorvastatin for three days increased plasma fibrin clot permeability (by 23%) and lysability (by 15–20%), independent of changes in lipid profile (69). These statin-induced alterations likely facilitate restoration of the patency of a thrombosed vein. Such modulation of fibrin structure and function is a novel antithrombotic effect of these drugs (70).

Factor V/Va

Thrombin is the predominant activator of FV, thus impaired thrombin generation will lead to reduced FV activation. At the site of microvascular injury, generation of FVa was delayed and the rate reduced by 19% after a three-month simvastatin therapy (41). The decreased rate of FVa formation was associated with a 26% lower rate of depletion of FV (41). These reductions were unrelated to simvastatin-induced changes in lipid profiles (41). After a 28-day atorvastatin administration in CAD patients, FV activation was reduced by 22% (43). Only three doses of 40 mg/day simvastatin led to a decrease in the maximum rates of FVa activation (by 20%) (45).

Factor XIII/XIIIa

Thrombin activates FXIII that cross-links fibrin monomers and binds antiplasmin to fibrin, increasing clot stability and resistance to lysis. Three-month simvastatin administration was found to be associated with 20% slower thrombin-induced FXIII activation and this effect is independent of cholesterol reduction; however, it was associated with decreased thrombin formation (41).

Thrombomodulin (TM)

The anticoagulant PC system, involving PC and TM is of crucial importance in haemostasis as evidenced by increased VTE risk in subjects deficient in PC, PS and FV^{Leiden} (66). Thrombin bound to

TM is anticoagulant, activating PC on endothelial cells. Activated PC (APC) inactivates FVa and FVIIIa, thus destroying the prothrombinase and intrinsic tenase complexes.

Available studies reported that statins at typical doses produce a slight decrease (10, 72) or no effect (73) on plasma soluble TM (72, 74). However, TM expressed on endothelial cells, not soluble TM, is of importance. Masamura et al. (75) were the first to show that pitavastatin can increase transcription of the TM gene and TM expression on endothelial cells. An inhibition of Rho protein, Rac/Cdc42, was reported to account for a concentration-dependent increase in TM antigen by statins, and this effect could be reversed with mevalonate or GGPP (75). Exposures to 10 μmol/l atorvastatin for 24 hours led to a 10-fold increase in TM transcripts, accompanied by a two- to three-fold increase in TM protein, cell surface TM antigen, and PC activation. Simvastatin and atorvastatin in cell culture counteracted tumour necrosis factor alpha (TNF-α)-induced down-regulation of endothelial TM expression associated with increased NO generation (76). Another mechanism involved in enhanced TM expression as well as decreased TF expression following simvastatin or lovastatin treatment (but not pravastatin) is increased expression of a transcription factor, Kruppel-like factor 2 (KLF2) (77). TM expression is modulated by shear stress. Rossi et al. (78) suggested an additive increase in KLF2 and TM expression, together with NO synthase expression in human abdominal aortic endothelial cells when simvastatin and low shear stress are combined. Simvastatin blocked suppression of endothelial TM and endothelial NO synthase (eNOS) expression induced by proinflammatory cytokines, irrespective of shear stress. Another statin, rosuvastatin, caused similar but weaker effects on endothelial cells (79).

The highest concentrations of TM are in the capillary bed, where the surface to volume ratio is greatest. In microvascular injury, but not in peripheral blood, we demonstrated that a three-day treatment with simvastatin resulted in enhanced APC inactivation of FVa (45). This effect appeared rapidly with no further increase after three months (41, 45).Faster FVa inactivation, following vascular injury (by 20%) was observed after 28 days of atorvastatin (43) or three days of simvastatin use (45).

Our data suggests that statins as shown for simvastatin given for three months can also stimulate an alternative pathway of FVa inactivation by increased generation of a 97-kDa fragment of the heavy chain by a probable cleavage at Arg643 in the presence of endothelial cells and/or platelets (41). Of note, three-days of simvastatin did not affect this process (45).

For an overview of effects of statins on the blood coagulation and anticoagulant pathways please see ►Table 1.

Other effects

Tissue factor pathway inhibitor (TFPI), a FXa-dependent inhibitor of the Ca^{2+}/FVIIa/TF complex, is bound to LDL (80%) and thus suggested to be affected by statins (►Figure 1). However, statins have been found to decrease total TFPI levels without reduction in free TFPI in hyperlipidaemic individuals or patients with CAD (31, 80, 81). Cumulative data indicate a negligible effect of statins on TFPI levels (82, 83).

Limited evidence suggests a slight reduction of FVIII coagulant activity during statin administration (84). In the MESA study involving middle-aged and elderly healthy individuals, statin users had significantly lower not only CRP but also FVIII (- 3%) as compared with non-users (57). Reduction in von Willebrand factor antigen associated with FVIII activity was reported following a long-term treatment with atorvastatin in stable CAD patients, however, other studies failed to confirm this observation (32, 72, 73).

There have been reports that statin administration slightly reduces plasma FVII antigen and/or coagulant activity with the largest effects noted in hyperlipidaemic subjects (85–87).

Table 1: Effects of statins on the blood coagulant and anticoagulant pathways.

Protein	Effects of statins	Experimental models	Signalling pathways	Evidence (References)
Tissue factor	Decreased expression	M, E, A, P	Rho/Rho kinase, NF-B, KLF-2, Akt	Established (13–18, 21–25, 27)
Thrombin	Decreased generation	E, M, A, P		Highly suggestive (27, 33,34,39–45, 49–55)
Fibrinogen	Decreased cleavage	A, P		Suggestive (41, 43)
Factor XIII	Decreased activation	P		Suggestive (41)
Thrombomodulin	Increased expression	E, P	Rho/Rho kinase, NF-B, KLF-2	Suggestive (70, 71, 75)
Factor Va	Increased inactivation	E, P		Inconsistent (41, 43)

M: monocytes/macrophages; E: endothelial cells; A: animal models; P: patient populations mostly at elevated cardiovascular risk.

Overall, effects other than reduced TF expression and up-regulation of TM expression most likely do not contribute to anticoagulant properties of statins *in vivo*. However, it should be acknowledged that over the last 20 years anticoagulant properties of statins were studied using multiple assays and coagulation models in various and rather small patient groups ranging from apparently healthy individuals to hyperlipidaemic patients in whom the effects are usually more potent. Some effects reported for instance changes in plasma fibrinogen concentrations were inconsistent which might indicate that some of the above effects might be coincidental. Further studies are needed to elucidate the actual statin-mediated effects on blood coagulation in various clinical settings and at various plasma cholesterol levels.

Clinical implications

Statins and arterial thrombosis

Several lines of evidence indicate that in addition to well-established benefits from statins in long-term secondary prevention of CAD, statin therapy is associated with a significant reduction of the risk of recurrent cardiovascular events during and following ACS (88). A meta-analysis of randomised controlled trials by Hulten et al. demonstrated that early, intensive statin therapy for ACS decreased the rate of death and cardiovascular events over two years of follow-up (hazard ratio [HR]: 0.81, 95% CI [confidence interval]: 0.77–0.87, p<0.001) (88). Survival benefit begins to occur between 4-12 months, achieving statistical significance by 12 months (88). However, statins initiated within 14 days after onset of ACS did not confer benefit in terms of hard clinical outcomes such as death or myocardial infarction (MI); risk ratios for the combined endpoint of death, MI, and stroke of early statin therapy compared to control were 0.93 (95% CI: 0.80–1.08; p=0.34) at one month and 0.93 (95% CI: 0.81–1.06; p=0.27) at four months following ACS (89). The current guidelines recommend the use of statin therapy before hospital discharge for all patients with ACS regardless of the baseline LDL cholesterol (90). In light of the current evidence, when blood coagulation and inflammatory processes are heavily activated, an early initiation of high-dose statin therapy in ACS has not been shown to reduce thrombotic events within the first four months despite additional statin-induced effects observed *in vitro* or under stable conditions (90). On the other hand, reduction in vascular outcomes in patients after four months of statin therapy since ACS cannot be explained by a relatively slow plaque stabilisation, therefore, the combined anticoagulant and antiplatelet effects of statins could be a major contributor, as suggested by a number of experimental studies (see above).

Statins and VTE

An obvious consequence of anticoagulant properties of statins could be a reduced incidence of pulmonary embolism (PE) and DVT that constitute VTE. Ray et al. (91) were the first to show that in a large cohort study, statins given at any dose are associated with 22% lower VTE risk compared with subjects not taking those agents (HR: 0.78, 95% CI: 0.69–0.87). Further studies yielded inconsistent data, including studies showing no benefits from statin use in terms of VTE prevention (92). Case-control studies of patients with prior VTE and healthy volunteers showed that in contrast to other hypolipaemic drugs, statin use can reduce by 45% the odds ratio (OR) of DVT (OR: 0.55, 95% CI: 0.46–0.67) (93, 94).

Cumulative analyses demonstrated that statins are able to decrease VTE risk, suggesting the class effect in this regard, regardless of age and the presence of cardiovascular disease (94). A meta-analysis of observational studies, that included four cohort studies and four case-control studies, demonstrated that statin administration is associated with lower VTE risk (OR: 0.67, 95% CI: 0.53–0.84), mainly due to lowered DVT risk (OR: 0.53, 95% CI: 0.22–1.29) (95). It should be noted that there is a weaker positive effect of statins in the elderly, as suggested in the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) performed in patients aged 70 to 82 years (96).

The randomised, double-blind, placebo-controlled Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) study demonstrated that 20 mg/day rosuvastatin reduces VTE risk in asymptomatic men 50 years of age or older and women 60 years of age or older, who had no evidence of cardiovascular disease and had LDL-cholesterol <3.4 mmol/l and CRP >2 mg/l at the initial visit (97). During a 1.9-year follow-up 34 rosuvastatin-treated individuals experienced DVT or PE vs 60 subjects receiving placebo (HR 0.57; 95% CI 0.37–0.86). Rosuvastatin led to the largest reduction in the rate of isolated DVT (by 55%) and failed to reduce PE (OR: 0.77, 95% CI 0.41–1.46) or death related to VTE (OR: 0.50, 95% CI 0.20–1.24). However, due to a low number of those episodes, this finding should be interpreted with caution. Of note, the risk of both idiopathic and provoked VTE in the rosuvastatin group was similar (39% vs 48%) (97). Until now, no randomised study has demonstrated similar effects of statins other than rosuvastatin.

Statins have been shown to reduce VTE risk in populations at particularly high thrombotic risk, namely in active cancer (OR: 0.33, 95% CI: 0.19–0.57) (98) and in nephrotic syndrome (HR: 0.2, 95% CI: 0.1–0.7) (99). Recently, a Dutch population-based registry of pharmacy records for patients hospitalised with an acute episode of PE showed that treatment with statins was associated with a reduced risk of recurrent PE (HR: 0.50, 95% CI: 0.36–0.70), both during and after discontinuation of oral anticoagulation, with the largest reduction in subjects receiving the most potent statins (100). However, evidence supporting benefits from statin therapy in terms of the VTE risk is inconsistent. In a large meta-analysis involving 22 trials of statin vs control (105,759 participants) and seven trials of an intensive vs a standard-dose statin regimen (40,594 participants) there was only a trend towards reduced risk of VTE (OR: 0.89, 95% CI: 0.78–1.01; p=0.08) with a similar impact on the risk of DVT and PE, regardless of a dose of the statin used (101). Some other studies failed to show even a trend towards lower VTE risk among statin users (102). In the light of inconsistent evidence of heterogeneous studies, it still remains to be deter-

mined which statins, at which doses and in which populations can be effective in reducing first-ever and recurrent VTE.

Statins and bleeding

One might suspect that anticoagulant properties of statins may be associated with increased bleeding risk as commonly seen during anticoagulation. Moreover, it has been suggested that cholesterol lowering may impair blood vessel integrity in the brain and cause small breaks in the walls of perforator arteries that branch orthogonally from major cerebral vessels, thus resulting in intracerebral haemorrhage, particularly when local activation of blood coagulation is suppressed. Indeed, a post-hoc analysis of a large, randomised trial in patients with cerebrovascular disease suggested increased risk for haemorrhagic stroke in patients receiving high-dose atorvastatin vs placebo (HR: 1.66, 95% CI: 1.08–2.55) (103).

A similar observation was made for subjects receiving simvastatin (104). It has been suggested that high statin dosages alone might predispose to cerebral haemorrhage. No increase in the rate of bleeds in subjects receiving rosuvastatin was observed in the JUPITER trial (97). A recent meta-analysis of 31 randomised controlled trials encompassing more than 91,000 statin-treated subjects demonstrated no difference in the incidence of intracranial haemorrhage between those individuals and controls (OR: 1.08, 95% CI: 0.88–1.32), while all-cause mortality (OR: 0.92; CI: 0.87–0.96; P=0.0007) was lower in subjects receiving statins (105). Recent studies have even suggested that simvastatin and atorvastatin treatment improves blood brain barrier integrity after experimental intracerebral haemorrhage (106). Current evidence speaks against an increase in the rate of bleeds among statin-treated subjects.

Conclusion

Experimental studies indicate that statins alter blood clotting at various levels. Statins produce anticoagulant effects predominantly via down-regulation of TF expression and enhanced endothelial TM expression resulting in reduced thrombin generation. Those effects are enhanced by profibrinolytic and antiplatelet effect of statins. Anticoagulant effects of statins are largest in subjects with hypercholesterolaemia; however, they are not associated with the magnitude of cholesterol reduction or duration of treatment as the anticoagulant effects of statins are observed as early as after 1–3 days of their administration.

It has been postulated that statin-induced anticoagulant effects can explain at least partially a reduction in VTE risk as shown in the JUPITER trial. It is tempting to speculate that statins could be effective also in secondary VTE prevention. However, it is still difficult to prove that the anticoagulant effects of statins translate into clinically relevant outcomes. 1) Since patients receiving statin therapy invariably have reduced blood cholesterol, the relative strength of anticoagulant effect compared to the lipid-lowering action is hard to assess. 2) Some effects of statins, including suppression of blood clotting, could be accounted for, to some extent, by cholesterol or oxLDL lowering. 3) Concentrations used to demonstrate the biologic effects of statins in some experimental studies, especially in inhibition of Rho geranylgeranylation, exceed those encountered in subjects receiving statins. However, *in vivo* human tissues are continuously exposed on statins. 4) There are differences in anticoagulant effects of various statins, for example pravastatin does not enhance TM expression and convincing evidence for VTE reduction refers solely to rosuvastatin. It remains to be determined whether all statins produce similar clinically meaningful anticoagulant effects beyond cholesterol lowering.

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Conflicts of interest

None declared.

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