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Atorvastatin favorably modulates proinflammatory cytokine profile in patients following deep vein thrombosis

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ABSTRACT

Background: Venous thromboembolism (VTE) has been shown to be associated with inflammation. Statins that might reduce VTE risk have been found to exert anti-inflammatory properties in patients at cardiovascular risk. We sought to investigate whether anti-inflammatory effects of atorvastatin can be observed in VTE patients.
Materials and Methods: Atorvastatin 40 mg/d was given for 3 days to 26 consecutive VTE patients following discontinuation of anticoagulant therapy and 25 controls. We evaluated interleukin (IL)-1b, IL-6, IL-8, IL-10, soluble P-selectin and von Willebrand factor (vWF) antigen in peripheral venous blood.
Results: The VTE patients displayed higher C-reactive protein ($p = 0.013$), IL-1b ($p = 0.03$), IL-8 ($p = 0.03$) and vWF ($p < 0.0001$) compared with the controls. In VTE patients atorvastatin decreased IL-6 ($p = 0.0003$), IL-8 ($p = 0.003$) and P-selectin ($p < 0.0001$), but increased IL-10 ($p = 0.001$), with no association with C-reactive protein or cholesterol-lowering effects. Atorvastatin reduced IL-1b ($p = 0.01$), IL-6 ($p = 0.03$) and P-selectin ($p = 0.002$) in controls. Residual venous thrombosis was associated with elevated IL-6 and P-selectin, whereas patients with proximal deep vein thrombosis showed elevated P-selecitn prior to and following statin administration (all $p < 0.05$).
Conclusion: A 3-day administration of atorvastatin reduces inflammation without decrease in C-reactive protein in VTE patients.

Introduction

Venous thromboembolism (VTE) is a major health problem with the average annual incidence of 1-2/1000 [1]. Growing evidence supports the hypothesis that inflammation is involved in the occurrence of VTE and its complications in part through secondary activation of blood coagulation associated with up-regulation of tissue factor [2], impairment of the endogenous fibrinolytic capacity [3] and platelet activation [4]. However, it is unclear whether low-grade systemic inflammation has relevant prothrombotic effects that might contribute to VTE. Interleukin (IL)-6 and IL-8 have been reported to be elevated in patients after their first thrombotic event who experienced recurrent VTE [5]. In a large cohort study, it has been shown that C-reactive protein (CRP) cannot predict future VTE [6]. Moreover, elevated concentrations of IL-6 and CRP in the acute phase of VTE have been shown to contribute to the post-thrombotic syndrome (PTS), a long-term complication [7]. On the other hand, it has been also demonstrated that infections increase the risk of VTE [8], providing additional evidence for close links between inflammatory state and thrombosis.

Statins, 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, have been shown to produce major anti-inflammatory effects in various clinical settings. The 2009 JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial showed a 43% decrease in DVT in association with reduced CRP levels in apparently healthy subjects with baseline CRP above 2 mg/l treated with rosuvastatin for almost 2 years [9]. However, a recent meta-analysis by Rahimi et al. [10] that included 29 clinical trials has not supported the view of a large protective effect of statins on VTE. Of note still a moderate reduction (up to 20%) in VTE risk with statin therapy cannot be ruled out. Based on randomized and cohort trials, there is evidence that subjects at increased cardiovascular risk with low-grade systemic inflammation, reflected by elevated CRP, represent a group that could benefit from anti-inflammatory effect of statins, regardless of baseline cholesterol levels [11]. In hypercholesterolemia, diabetes or coronary artery disease (CAD), statins can decrease circulating concentrations of proinflammatory mediators, including IL-1b, IL-6, IL-8, P-selectin [12,13] and increase anti-inflammatory IL-10 [14]. von Willebrand factor (vWF), a marker of endothelial damage, is largely unaltered by statin administration [15]. On the other hand, HMG-CoA reductase inhibitors have anticoagulant properties including reduced tissue factor expression, increased thrombomodulin expression and favorable alterations to fibrin clot structure/function [16]. Recently, we have demonstrated that atorvastatin given for 3 days improves

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plasma fibrin clot lysability in subjects following VTE [17]. It is unclear whether statins other than rosuvastatin are potent enough to affect proinflammatory mediators in subjects following VTE. We tested the hypothesis that short-term atorvastatin administration to VTE patients can favorably alter the profile of circulating inflammatory mediators.

Materials and Methods

Participants

We studied 26 patients who developed first-ever documented VTE and completed anticoagulation 6 months or more prior to enrollment and 25 controls without history of VTE or venous insufficiency matched for age, sex and cardiovascular risk factors. Signs or symptoms of CAD, diabetes, hemorrhagic diathesis, autoimmune diseases, hypo- or hyperthyroidism, acute inflammation (CRP >10 mg/l), use of any medication (apart from antihypertensive drugs) and severe comorbidities e.g. cancer were the exclusion criteria for both groups.

Overweight was defined as body mass index (BMI) of 25–29.9 kg/m^2. Arterial hypertension was diagnosed in subjects with diastolic blood pressure above 90 mmHg, but less than 100 mmHg and/or systolic pressure in the range of 140-160 mmHg at least 2 times or when individuals were treated with antihypertensive medications. Hypertensive subjects with mild arterial hypertension were eligible. Current smoking was defined as smoking of 5 or more cigarettes daily. Family history of VTE or CAD was established when at least one first-degree relative of a subject declared a previous documented episode of any of these diseases. Patients with VTE who withdrew anticoagulation at least one month prior to the enrollment were eligible. The diagnosis of deep vein thrombosis (DVT) was established by a positive finding of colour duplex sonography. Proximal DVT was defined as thrombosis involving the popliteal or more proximal deep vein segments. Residual venous thrombosis (RVT) was defined as >20% intraluminal stenosis [18] and was assessed at the enrollment to the study by the same physician using the same ultrasound apparatus (GE Vivid 7, USA). Patients with DVT were assessed after the study by the same person using the Villalta scale and a score of 5 or more indicated the presence of PTS [19].

The University Ethical Committee approved the study, and patients provided written, informed consent.

The VTE patients and controls received atorvastatin (Sortis, Pfizer) 40 mg/day taken in the evening for three days on an open-label basis.

Blood Collection and Biochemical Analysis

Fasting blood samples were collected at 8–10 AM before the first dose, and then after three doses of atorvastatin in the fourth morning. Lipids, CRP, alanine aminotransferase and fibrinogen were measured using automated analyzers (Sysmex, Japan and Cobas 6000, Roche, Switzerland). Plasma and serum aliquots were centrifuged and stored in aliquots at -80 °C to allow batch analysis. Levels of vWF antigen (vWF:Ag; Dako, Glostrup, Denmark), soluble P-selectin, IL-1b, IL-6, IL-8 and IL-10 (all R&D Systems, Abington, UK) were measured in plasma using commercially available enzyme-linked immunosorbent assays according to the manufacturers' instructions.

Statistical Analysis

The relationships between categorical variables were analyzed by chi^2 test. Continuous variables, presented as median (interquartile range), were compared between the groups by Mann-Whitney U test and between the time points using Wilcoxon test. Correlations were analyzed by Spearman's rank coefficient. The comparison of IL-1b, IL-6, IL-8, IL-10, P-selectin and vWF between cases and controls were tested also with the adjustment for body mass index (BMI) using multiple linear regression. Variables having positively skewed distribution were approximated to normality by square root transformation before entering the model. A p-value of $\leq$0.05 was considered statistically significant.

Results

Baseline Data

The VTE and control groups were similar in regard to demographics (Table 1). Nine VTE patients (35%) had unprovoked VTE. Proximal DVT was diagnosed in 16 (61%). The presence of RVT in the lower extremity deep veins was observed in 8 (31%) subjects and PTS was diagnosed in 14 (54%) patients. Family history of VTE and overweight were more common in VTE patients. The mean duration of anticoagulation was 7 months (range, 5-12 months) and time from discontinuation of anticoagulation was 8 months (range, 3.5-15 months).

The VTE patients displayed higher levels of CRP ($p = 0.013$), IL-1b ($p = 0.03$), IL-8 ($p = 0.03$) and vWF ($p < 0.0001$) compared with the controls (Table 2). Fibrinogen, IL-6, IL-10 and P-selectin were similar in both groups (Table 2). Comparisons of IL-1b, IL-6, IL-8, IL-10, P-selectin and vWF between cases and the controls were not affected by the adjustment for BMI (Suppl. Table 1). Before atorvastatin administration in VTE patients IL-6 was positively correlated with CRP ($R = 0.47$, $p = 0.015$) and P-selectin ($R = 0.51$, $p = 0.007$). Moreover, IL-1b was correlated with CRP ($R = 0.44$, $p = 0.02$). There were inverse correlations of IL-1b with time since VTE episode ($R = -0.75$, $p < 0.001$) and time since discontinuation of anticoagulation ($R = -0.84$, $p < 0.001$) and also P-selectin with age ($R = -0.54$, $p = 0.004$). Other inflammatory mediators did not show similar associations (data not shown).

Statin-induced Alterations

A 3-day atorvastatin administration was uneventful. In both groups atorvastatin resulted in reductions in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (Table 2). No statin-induced changes in alanine aminotranspherase, CRP and fibrinogen were observed in either group.

As presented in Table 2, in VTE patients atorvastatin reduced IL-6 (by 9.8%), IL-8 (by 2.4%) and P-selectin (by 18.7%), but increased IL-10 (by 8.2%). After atorvastatin CRP, fibrinogen, IL-1b and vWF remained unaltered. In controls atorvastatin decreased IL-1b (by 7.2%), IL-6 (by 7.3%) and P-selectin (by 6.2%) but did not change CRP, fibrinogen, IL-8, IL-10 or vWF.

In VTE patients post-treatment IL-6 was positively correlated with CRP ($R = 0.54$, $p = 0.004$) and P-selectin ($R = 0.47$, $p = 0.01$) After atorvastatin there were inverse correlations of IL-6 with IL-10 ($R = -0.41$, $p = 0.04$) and IL-6 with age ($R = -0.45$, $p = 0.02$) and also vWF with P-selectin ($R = -0.45$, $p = 0.02$) and vWF with CRP ($R = -0.41$, $p = 0.04$). Of the circulating mediators only post-treatment P-selectin showed correlations with TC ($R = -0.46$, $p = 0.02$) and LDL-C (β=-0.42, $p = 0.03$) and vWF with high-density lipoprotein cholesterol ($R = 0.45$, $p = 0.02$). There were no correlations between changes in lipids and the mediators measured in blood following a 3-day atorvastatin administration in VTE patients or in controls (data not shown).

Table 1
Characteristics of the controls and deep vein thrombosis (DVT) patients.

	Controls (n = 25)	DVT patients (n = 26)	P
Age, years	43 (34-50)	44 (33-53)	0.84
Male, n (%)	14 (56)	14 (54)	0.9
BMI, kg/m^2	24.4 (23.0-27.8)	29.2 (26.2-30.5)	0.02
Current smoking, n (%)	6 (24)	9 (35)	0.52
Family history of CVD, n (%)	5 (20)	6 (23)	0.86
Family history of DVT, n (%)	0 (0)	4 (15)	-
Arterial hypertension, n (%)	4 (16)	5 (19)	0.85

Abbreviations: BMI, body mass index; CVD, cardiovascular disease.