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Regular Article

Atorvastatin has antithrombotic effects in patients with type 1 diabetes and dyslipidemia ☆

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ABSTRACT

Introduction: Diabetes is a prothrombotic state involving a more thrombogenic fibrin network. In the present study we investigated the effects of lipid-lowering therapy with atorvastatin on fibrin network structure and platelet-derived microparticles in patients with type 1 diabetes and dyslipidemia.

Materials and Methods: Twenty patients were treated with atorvastatin (80 mg daily) or placebo during 2 months in a randomized, double-blind, cross-over study. Fibrin network permeability, expression of glycoprotein IIIa, P-selectin and tissue factor on platelet-derived microparticles, plasma endogenous thrombin potential, plasminogen activator inhibitor-1 and tissue plasminogen activator antigen levels were assessed. Additionally, levels of plasma fibrinogen, high-sensitivity C-reactive protein and glycated haemoglobin were measured.

Results: During treatment with atorvastatin, fibrin network permeability increased ($p = 0.01$), while endogenous thrombin potential and expression of glycoprotein IIIa, P-selectin and tissue factor decreased ($p < 0.01$). *In vitro* experiments indicated that platelet-derived microparticles influence the fibrin network formation as fibrin network permeability decreased significantly when platelet-derived microparticles were added to normal plasma. Baseline levels of plasminogen activator inhibitor-1 and tissue plasminogen activator antigen as well as plasma fibrinogen and high-sensitivity C-reactive protein were within reference values and not significantly changed during atorvastatin treatment, while glycated haemoglobin increased 0.3% ($p < 0.001$).

Conclusions: Novel treatment effects were found in patients with type 1 diabetes and dyslipidemia during atorvastatin therapy, i.e. a more porous fibrin network, to which reduced expression of glycoprotein IIIa, P-selectin and tissue factor on platelet-derived microparticles may contribute. The observed impairment of glycemic control during long-term statin treatment deserves attention.

Diabetes is considered to be a prothrombotic state to which factors such as increased platelet adhesion and aggregation [1], elevated levels of plasma fibrinogen [2,3], and increased thrombin generation [3] may contribute. Upon activation with thrombin, fibrinogen forms fibrin monomers, which in the presence of FXIII polymerize and form a cross-linked fibrin network [4]. The fibrin network is influenced by the environment in which it is formed and the constituents present in plasma, and may vary between a dense structure made up of thin fibers and a porous fiber conformation with thicker fibers [5,6]. The structure of the fibrin network influences the fibrinolytic rate [5,7], and a dense fibrin conformation is considered to be thrombogenic, since it is less susceptible to fibrinolysis, whereas a more porous fibrin network is associated with increased fibrinolytic capacity [5,7]. In our previous studies we have shown that patients with type 1 diabetes have a less permeable fibrin network [8]. When the fibrin network was studied along with improvement of metabolic control, positive changes in the fibrin network seemed mainly to be related to improved lipid levels [9]. These findings indicated that dyslipidemia in patients with type 1 diabetes may influence the fibrin network and suggest that treatment with lipid-lowering drugs may be beneficial in this respect. Consequently, the primary aim of the present study was

Abbreviations: PDMPs, Platelet-derived microparticles; Ks, fibrin network permeability coefficient; GP, glycoprotein; TF, tissue factor; ETP, endogenous thrombin potential; hsCRP, high-sensitivity C-reactive protein; HbA_{1C}, glycated haemoglobin; CVD, cardiovascular disease; MESF, molecules of equivalent soluble fluorochrome; TRAP, thrombin receptor activator peptide; C, cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; F1+2, prothrombin fragment 1+2; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activator.

☆ The results of this study were presented at the International Society on Thrombosis and Haemostasis in Boston, 2009-07-15.

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to investigate the effect of the lipid lowering drug atorvastatin on fibrin network permeability in patients with type 1 diabetes and dyslipidemia.

Beneficial effects of lipid-lowering therapy with statins in patients with cardiovascular disease (CVD) have indeed been shown in numerous studies. Additionally, reduced occurrence of symptomatic venous thromboembolism was recently shown during treatment with rosuvastatin in the JUPITER trial in healthy subjects with normal LDL-cholesterol levels [10]. Several recent large scale trials have shown the efficacy of statin therapy in primary prevention of CVD in patients with type 2 diabetes [11,12], whereas the long-term effects of statins in type 1 diabetic patients have not been evaluated. Importantly, statins seem to exert potential antithrombotic effects that are independent of their lipid-lowering properties, including reductions in thrombin generation and platelet activation [13,14]. Increased platelet activation has been observed in patients with type 1 diabetes [15]. Upon activation, platelets shed small particles (0.1-1 μm in size) into the circulation. These platelet-derived microparticles (PDMPs) are membrane buds which express various surface antigens, including glycoprotein (GP) IIIa, P-selectin and tissue factor (TF). PDMPs may be involved in hemostasis and thrombosis, and experimental studies have shown that PDMPs may bind to fibrin and act as procoagulants [16]. Reduced GPIIIa exposure on PDMPs and decreased expression of TF on human monocytes have been reported during statin therapy [13,17]. We have previously found increased expression of P-selectin and TF on PDMP in type 1 diabetes [18], and in the present study we were interested to see if statin therapy can affect expression of GPIIIa, P-selectin and TF on PDMP in patients with type 1 diabetes (secondary variables).

Materials and Methods

Study design

A double-blind, cross-over study with randomization to receive a daily dose of 80 mg atorvastatin or matched placebo for 2 months was designed (Fig. 1). Investigations were performed at the start and at the end of the treatment periods, which were separated by a wash-out period of 2 months.

Patients

Twenty patients were recruited from the Department of Endocrinology and Diabetology, Danderyd University Hospital, Stockholm. Eligible for the study were patients between the ages of 30–70 years with type 1 diabetes and elevated levels of LDL-cholesterol (>2.5 mmol/L) and/or total cholesterol (>4.5 mmol/L). Patients with a history of macrovascular events were excluded in order to avoid the influence of macrovascular disease and vasoactive medications on the investigated variables. All patients arrived at the laboratory between 8 and 9 a.m. after a 10-hour fast. Clinical signs of peripheral neuropathy in the feet were investigated with tests of vibration and superficial sensation using vibration fork (128 Hz) and monofilament (Semmes-Weinstein 5.07), respectively. Albuminuria was investigated by urinary dipstick tests (Clinitek®, Bayer HealthCare LLC), and peripheral arterial occlusive disease was assessed by determination of the toe/arm blood pressure index.

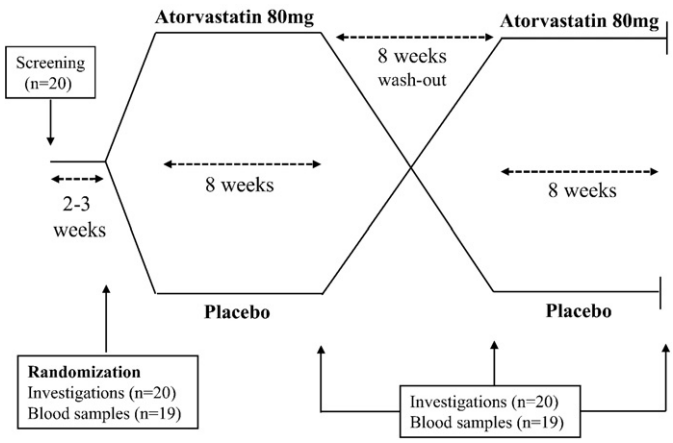

Fig. 1. Cross-over study in 20 patients with type 1 diabetes randomized to treatment with atorvastatin and placebo for 2 months, respectively, and with a wash-out period of 2 months in-between the treatments.

Fibrin network

Fibrin network structure was studied *in vitro* in citrated plasma samples by measurement of the permeability coefficient (Ks), as described in detail elsewhere [4,19]. In brief, plasma samples were dialyzed, centrifuged and supplemented with $CaCl_2$ and thrombin to final concentrations of 20 mmol/L and 0.2 NIH/mL, respectively. Buffer was percolated through the formed fibrin clots, after which Ks was calculated. Low levels of Ks indicate reduced fibrin network permeability [4,6,20]. The inter-assay coefficient of variation (CV) was 9.5%.

Platelet derived microparticles

The method has been described in detail recently [18]. In brief, a microparticle-enriched pellet was obtained from plasma samples and incubated with CD42a-PE (Glycoprotein IX, BD, Clone Alma-16), together with either CD61-FITC (GPIIIa, AbD Serotec, Clone Y2/51), CD62P-FITC (P-selectin, AbD Serotec, Clone AK-6) or CD142-FITC (TF, AbD Serotec, Clone CLB/TF-5). The PDMP-gate was determined using forward scatter and CD42a expression. Expression of GPIIIa, P-selectin and TF was measured using a flow cytometric assay, where mean fluorescence intensities (MFI) of antigen positive particles were translated into MESF-values (Molecules of Equivalent Soluble Fluorochrome) [19]. Reproducibility experiments using plasma samples from healthy individuals showed that the CV for MESF of CD62P and CD142 (both intra- and interassay) was <10% [18].

Thrombin generation

A calibrated, automated thrombogram assay was performed as described by Hemker et al. [21] and according to the manufacturers´ instruction (Thrombinoscope BV, Maastricht, the Netherlands). The calculated area under the curve (endogenous thrombin potential, ETP) represents the total amount of thrombin generated over time.

In vitro experiments

In vitro experiments were performed to investigate the possible association between PDMPs and fibrin network permeability. In order to obtain high concentrations of PDMPs, platelet rich plasma samples from healthy subjects were stimulated with thrombin receptor activator peptide (TRAP) 6 (Trio-lab, Gothenburg, Sweden; final concentration of 32 μmol/L). A PDMP-enriched pellet was obtained through high-speed centrifugation [18]. The expression of GPIIIa, P-selectin and TF on PDMPs after stimulation with TRAP was 10607 ± 1039, 4675 ± 1476 and 2898 ± 1294 MESF, respectively. The pellet was added to normal pooled plasma (fibrinogen concentration 2.6 g/L; $n = 6$) or commercial plasma (Haemochrom, Diagnostica AB; fibrinogen concentration 3.16 g/L; $n = 2$), together with $CaCl_2$ (20 mmol/L) and TRIS buffer. The permeability coefficients of the fibrin clots were determined as previously described [4,19]. Fibrin clots made without PDMP-enriched pellets served as controls.