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Dyslipidemia shifts the tissue factor/tissue factor pathway inhibitor balance toward increased thrombogenicity in atherosclerotic plaques Evidence for a corrective effect of statins

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Abstract

Background: Tissue factor (TF) is a key mediator of atherosclerotic plaque thrombogenicity and may be regulated by plaque TF pathway inhibitor (TFPI). High atherogenic lipoproteins are a well-known arterial risk factor, but their effects on the TF/TFPI balance in atherosclerotic plaques, as well as those of widely used lipid-lowering agents such as statins, are incompletely understood.
Objectives: We analyzed the TF/TFPI balance in carotid plaques from 86 patients, according to the presence of dyslipidemia and statin therapy. *Results:* In patients with untreated dyslipidemia (ApoB/ApoA1 ratio >0.7) (D+) ($n = 44$), TF antigen (TF) tended to be higher than in those without dyslipidemia (D−) ($n = 16$). In patients with statins (S+) ($n = 26$), TF was lower than in D+ ($p = 0.02$) and similar to that of D− patients. TFPI antigen was higher in D− than in D+ and S+ patients ($p \leq 0.02$). As a result, the TF/TFPI (mol/mol) ratio was higher in D+ than in D− or S+ patients ($p \leq 0.005$). TF activity correlated to TF/TFPI ratio ($p < 0.0001$), and was higher in the D+ than in the D− and in the S+ patients ($p = 0.02$). Among analyzed clinical risk factors and biological parameters, including CRP, dyslipidemia was the only independent predictor for low plaque TFPI and high TF/TFPI ratio. Histochemistry showed that TF and TFPI were mainly expressed in macrophage-rich regions surrounding the lipid-rich core in the three groups.
Conclusions: These results indicate that dyslipidemia is associated with a shift of the TF/TFPI balance and of TF activity toward higher plaque thrombotic potential. Statins correct this equilibrium mainly by decreasing plaque TF together with blood atherogenic lipoproteins.

Keywords: Tissue factor; Tissue factor pathway inhibitor; Atherosclerosis; Statin

Acute cardiovascular events are mainly mediated by thrombosis on disrupted atherosclerotic plaques. Increased blood atherogenic lipoprotein levels are a well-known risk factor for atherosclerosis progression, and also for acute cardiovascular events. Dyslipidemia, defined as an increased apolipoprotein B/apolipoprotein A1 ratio (ApoB/ApoA1 ratio) was recently demonstrated to be the most relevant risk factor in large epidemiological studies [1,2]. However, the relationships between dyslipidemia and plaque thrombogenicity are not fully understood. Tissue factor (TF), the principal initiator of blood coagulation after binding to

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activated factor VIIa (FVIIa), is present in atherosclerotic plaques, especially in advanced or unstable ones, where it seems to be an important contributor to plaque thrombogenicity [3–5]. TF-initiated coagulation is regulated by a specific inhibitor, the TF pathway inhibitor (TFPI), which is also expressed in the atherosclerotic vessel wall [6–9]. The possibility that plaque TF activity may be regulated by TFPI within atherosclerotic plaques has been suggested by several independent observations. In *ex vivo* models of atherosclerotic lesions and in experimental models, TF-mediated thrombosis was reduced by TFPI inhibition [10–12]. In human plaques, TF activity was found to be higher in plaques that displayed less TFPI, and preincubation with anti-TFPI antibody enhanced TF-mediated coagulation activation [6,9]. Therefore, the balance between TF and TFPI may be of key importance in plaque thrombogenicity.

In experimental models, it is well demonstrated that high cholesterol diet upregulates and lipid-lowering downregulates TF in atherosclerotic plaques [13–15]. *In vitro*, oxidized lipids and free cholesterol upregulate TF expression in monocyte-derived macrophages [16,17]. Moreover, lipid-lowering agents such as statins, which are increasingly used to reduce atherosclerosis progression and acute ischemic events, also downregulate TF expression [18], an effect which is generally considered as a non-lipid dependent, pleiotropic one [13,19,20].

By contrast, the variations of TFPI in atherosclerotic plaques are far less well known. At the present time, little is known on the relationships between dyslipidemia and TFPI levels in atherosclerotic plaques. Moreover, though statins are known to decrease plasma TFPI [18,21], their effect on plaque TFPI has been poorly investigated.

To test the hypothesis that dyslipidemia and statin therapy could influence the TF/TFPI balance in atherosclerotic plaques through distinct variations of plaque TF and TFPI levels, we analyzed the respective levels of plaque TF and TFPI, and TF activity in carotid endarterectomy plaques from 86 patients, according to dyslipidemia, and the use of statin therapy.

1. Materials and methods

1.1. Study population

Eighty-six consecutive patients (65 men, age 70 ± 0.8 years, mean $\pm$ S.E.), eligible for surgical carotid endarterectomy according to the European Carotid Trialists' Collaborative Group [22] were included in the study. Informed consent was obtained from all patients. Cardiovascular risk factors were recorded at the time of surgery. Acute ischemic cerebral event (ICE) before surgery were assessed through a retrospective blinded analysis of the medical files by a neurologist (CL). Dyslipidemia was defined as an ApoB/ApoA1 ratio >0.7. Hypertension was defined as a systolic blood pressure $\geq$140 or diastolic $\geq$90 mm Hg, diabetes mellitus as a fasting plasma glucose $\geq$1.26 g/L, obesity as a body mass index (BMI) value >25 kg m^{-2}. Patients were categorized as smokers if they were current smokers or had stopped smoking for less than 1 year. Antiplatelet and statin therapy were recorded.

1.2. Determination of lipid, coagulation and inflammation blood parameters

Blood sampling was performed the day before surgery (non-anticoagulated blood for lipid and inflammatory parameters, blood anticoagulated with 3.8% trisodium citrate, 9:1 (vol/vol) for coagulation parameters) and stored at −80 °C after centrifugation.

ELISA kits were used for the determination of thrombin-antithrombin (TAT) complexes (Dade Behring, France) and interleukin 6 (IL-6) (R&D Systems, UK). Fibrinogen was measured using a clotting assay (Biomerieux, France), C-reactive protein (CRP) and apolipoproteins B and A1 by immunonephelemetric assays (Behring, France and Boehringer, Germany, respectively). Cholesterol levels were determined with an enzymatic kit (Boehringer, Germany).

1.3. Endarterectomy specimen processing

Immediately after endarterectomy, plaques were rinsed with saline and macroscopically classified as without or with a "predominant lipid-rich core" when the lipid-rich core represented more than 50% of their total weight according to Stary classification [23]. A representative part of the specimen was sampled for histochemical analysis, and the remaining portion was homogenized in extraction buffer (20 mmol/L Tris–HCl, 0.5% of non-ionic detergent Nonidet NP40) at 24,000 rpm for 1 min with a high-speed homogenizer (Ultra-Turrax T25 Ika-Labortechnik, Germany) and then agitated at 4 °C (100 mg of plaque in 500 μL extraction buffer) [24]. As a preliminary step, we analyzed the kinetics of protein extraction after 1–12 h of stirring. The protein concentration was maximal after 1 h and did not differ afterwards, thus plaque extracts were subsequently obtained after 1 h stirring. After centrifugation (3300 × *g*, 15 min, 4 °C) the supernatants were frozen at −80 °C.

1.4. Protein and cholesterol determination in plaque homogenates

Protein concentrations in homogenates were determined by turbidimetry at 650 nm after precipitation with sulfosalicylic acid. Plaque total cholesterol (free and esterified forms) was measured using the Zak chemical method [25]. Cholesterol level was expressed as mg/g of protein.

1.5. TF and TFPI determinations in plaque homogenates

The cofactor activity of TF for FVIIa (subsequently referred to as TF activity) was measured using a two-step chromogenic assay, as previously described, using purified