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Relationship between ADAMTS13 activity, von Willebrand factor antigen levels and platelet function in the early and late phases after TIA or ischaemic stroke

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ABSTRACT

Background: Reduced ADAMTS13 activity is seen in thrombotic thrombocytopenic purpura (TTP), and may lead to accumulation of prothrombotic ultra-large von Willebrand factor (ULVWF) multimers *in vivo*. ADAMTS13 activity and its relationship with VWF antigen (VWF:Ag) levels and platelet function in 'non-TTP related' TIA or ischaemic stroke has not been comprehensively studied.
Methods: In this prospective pilot observational analytical case–control study, ADAMTS13 activity and VWF:Ag levels were quantified in platelet poor plasma in 53 patients in the early phase (≤4 weeks) and 34 of these patients in the late phase (≥3 months) after TIA or ischaemic stroke on aspirin. Data were compared with those from 22 controls not on aspirin. The impact of ADAMTS13 on platelet function in whole blood was quantified by measuring Collagen-ADP (C-ADP) and Collagen-Epinephrine closure times on a platelet function analyser (PFA-100®).
Results: Median ADAMTS13 activity was significantly reduced in the early phase (71.96% vs. 95.5%, $P < 0.01$) but not in the late phase after TIA or stroke compared with controls (86.3% vs. 95.5%, $P = 0.19$). There was a significant inverse relationship between ADAMTS13 activity and VWF:Ag levels in the early phase ($r = -0.31$; $P = 0.024$), but not in the late phase after TIA or stroke ($P = 0.74$). There was a positive correlation between ADAMTS13 activity and C-ADP closure times in early phase patients only, likely mediated via VWF:Ag levels.
Discussion: ADAMTS13 activity is reduced and VWF:Ag expression is increased within 4 weeks of TIA or ischaemic stroke onset, and can promote enhanced platelet adhesion and aggregation in response to stimulation with collagen and ADP via VWF-mediated pathways. These data improve our understanding of the dynamic haemostatic and thrombotic profiles of ischaemic cerebrovascular disease (CVD) patients, and are important in view of the potential future role that ADAMTS13 may have to play as an anti-thrombotic agent in CVD.

1. Introduction

Von Willebrand factor (VWF) is a multimeric plasma glycoprotein synthesised in vascular endothelial cells and megakaryocytes [1,2]. VWF is stored as a mixture of multimers in the α-granules of platelets, and as ultra-large multimers in Weibel–Palade bodies of endothelial cells [3]. After release in response to injury or inflammation, VWF may bind to GP1b-IX-V or αIIbβ3 receptors on platelets, and promote platelet adhesion, aggregation and subsequent thrombus formation. If not consumed immediately, ultra-large VWF is cleaved by **AD**isintegrin-like **A**nd **M**etalloprotease with **T**hrombo**S**pondin type I repeats **13** (ADAMTS13) into smaller, less adhesive multimers that circulate in plasma [2]. Deficiency of ADAMTS13 activity is observed in thrombotic thrombocytopenic purpura (TTP) [4], and is associated with accumulation of prothrombotic ultra-large von Willebrand factor (ULVWF) multimers. Murine stroke models have suggested that reduced ADAMTS13 activity significantly aggravates ischaemic brain injury [5,6]. A number of studies have identified either low ADAMTS13 antigen levels or activity in coronary artery disease [7–9] or at a single timepoint after ischaemic

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stroke [10–12], but other studies have not confirmed these findings [1]. Increased VWF levels have been reported in ischaemic cerebrovascular disease (CVD) patients [1,13–15], and may cause platelet hyper-reactivity and shortened 'closure times' on a high shear stress-dependent platelet function analyser called the PFA-100® [16–18]. ADAMTS13 activity has been shown to be inversely correlated with VWF antigen levels [19], but to our knowledge, the impact of ADAMTS13 activity on platelet function on the PFA-100® in patients in both the early and late phases after TIA and stroke has not been studied.

The aims of this study were to assess ADAMTS13 activity in both the early and late phases after 'non-TTP related' TIA or ischaemic stroke, to assess the relationship between ADAMTS13 activity and VWF antigen (VWF:Ag) levels in CVD patients, and to determine whether ADAMTS13 activity independently influences platelet reactivity in CVD. We hypothesised that ADAMTS13 activity would be reduced in CVD patients vs. controls, and that ADAMTS13 activity would correlate with VWF:Ag levels and platelet reactivity in ischaemic CVD.

2. Methods

2.1. CVD patient inclusion criteria

Consecutive eligible patients referred by General Practitioners or Consultant colleagues to our secondary and tertiary referral stroke prevention outpatient clinic or inpatient stroke neurology or liaison neurology service at the National Hospital for Neurology and Neurosurgery, University College London Hospitals, UK were recruited to this prospective pilot observational analytical case–control study. Patients were included if they were older than 18 years of age, had experienced a TIA or ischaemic stroke within the preceding 4 weeks (early phase), had been commenced on aspirin, and were likely to be available for clinical and laboratory follow-up at least 3 months after symptom onset (late phase). The study was approved by the Local Research Ethics Committees of the participating Hospitals. Written informed consent (or written assent, where appropriate) was obtained in all cases.

2.2. CVD patient exclusion criteria

We excluded patients who were on another antiplatelet or non-steroidal anti-inflammatory drug in combination with aspirin, or those receiving heparin or warfarin. Patients were also excluded if they had a history of primary intracerebral haemorrhage, myocardial infarction within the preceding 3 months, on-going unstable angina, unstable symptomatic peripheral vascular disease, major surgery or systemic haemorrhage within the preceding 3 months, or if they had systemic vasculitis, underlying neoplasia, or a known bleeding or clotting diathesis, including TTP.

One examiner (DJHM) clinically assessed all subjects at each timepoint, and information regarding vascular risk factors, smoking status, alcohol intake, and medication use was collected prospectively. Results of routine haematological, coagulation, biochemical and blood glucose testing were collected prospectively. All CVD patients had a brain CT or MRI, and colour Doppler ultrasound examination of carotid[18] and vertebral arteries. Some patients underwent extra- or intra-cranial magnetic resonance angiography or intra-arterial catheter angiography, if deemed appropriate by the treating physician. A chest radiograph and an electrocardiograph (ECG) were obtained in all patients. A 24-hour ECG recording was performed if paroxysmal atrial fibrillation or flutter was suspected clinically, and transthoracic ± transoesophageal echocardiography was also carried out if a cardioembolic cause for stroke or TIA was suspected, or when other investigations were uninformative, as previously described [18]. The underlying mechanism responsible for the TIA or ischaemic stroke was categorised according to slight modifications of the TOAST classification as large artery atherosclerotic, lacunar, cardio-embolic, other determined, and undetermined aetiology (Table 2) [20]. For the purpose of this study, large artery atherosclerotic TIA or stroke specifically referred to TIA or stroke in the vascular territory supplied by a severe (>70%) ipsilateral extracranial carotid stenosis or occlusion to comply with inclusion criteria for other ongoing collaborative studies in our laboratory. The late phase follow-up in the large artery atherosclerotic subgroup was performed ≥3 months after carotid surgery or endovascular treatment, unless this treatment had been delayed for at least 3 months after the initial event.

2.3. Control subjects inclusion and exclusion criteria

Control subjects were recruited from the staff at The Haemostasis Research Unit, University College London Hospitals, from the local population, and from amongst spouses of patients and control subjects. Subjects were excluded from the control group if they had a history of known vascular disease, or evidence of asymptomatic severe (>70%) carotid or vertebral artery stenosis on colour Doppler ultrasound imaging. Otherwise, the exclusion criteria were identical to those applied to the patient group. It was expected that control subjects would have a different vascular risk profile to the patient population.

2.4. Blood sampling and laboratory tests

All subjects were rested for at least 20 min before venepuncture, and free-flowing blood was collected using a 21-G Butterfly needle (Venisystems™, Abbott, Ireland) and a Vacutainer® system with a luer adaptor (Becton-Dickinson Vacutainer Systems, UK). For all studies, the tourniquet was released during collection of the first 4 ml of blood into a sterile Vacutainer tube containing 7.2 mg of K_2 EDTA or 0.054 ml of 15% K_3 EDTA. This sample was used for measurement of a full blood count (FBC). Four further 4.5 ml samples were collected into sterile Vacutainer tubes containing 0.5 ml of 3.2% buffered sodium citrate. The first 4.5 ml citrate-anticoagulated whole blood sample was used for measurement of platelet reactivity and closure times on the PFA-100®in response to stimulation with Collagen-ADP (C-ADP) and Collagen-Epinephrine (C-EPI) between 2 and 2.5 h after venepuncture, as previously described [18]. The maximum closure time recorded by the device is 300 s, and we arbitrarily defined closure times >300 s as 301 s [18].

The next two citrated samples were used to prepare double-centrifuged (2000 g × 15 minutes) platelet-poor plasma (PPP) that was immediately stored at −70 °C for later analysis. VWF antigen levels were measured in PPP with an automated latex agglutination assay (STA Liatest VWF, DiagnosticaStago, Asnsieres, France), modified for use on a Sysmex CA-1500™ fully-automated coagulometer (Sysmex UK Ltd, Milton Keynes, UK) using Coagulation Reference Plasma (Technoclone, Vienna, Austria) to prepare a standard curve [18].

ADAMTS13 activity in double-centrifuged PPP was quantified using a collagen binding assay described by Yarranton et al., and modified from Gerritsen et al. [21,22]. Samples were analysed in batches at the end of the study period, with no sample stored for longer than 72 months. Freeze-thawing of samples was avoided by storing samples in multiple, separate aliquots. After dilution of the plasma, ADAMTS13 was activated by incubation at 37 °C for 5 min with 10 mmol/L barium chloride and 0.9 mmol/L Pefabloc SC® (Sigma-Aldrich, Poole, UK). Immediately after activation, VWF concentrate (final concentration = 0.561 IU/mL; French Laboratory of Fractionation and Biotechnology, Lille, France) was added and the samples incubated overnight in the presence of urea (final concentration = 1.36 mol/L) at 37 °C. The reaction was stopped by adding disodium-EDTA (final concentration = 0.1 mol/L). Samples were then diluted and added to a collagen-coated microtitre plate. The residual high molecular weight multimers of VWF bound to collagen on the plates and were detected with an anti-VWF antibody conjugated to HRP (Dako), followed by detection with 1,2-ortho-phenylenediamine substrate (Sigma-Aldrich). The results were expressed relative to the ADAMTS13 activity of pooled normal