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Regular Article

Increased thrombin generation in inflammatory bowel diseases

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ABSTRACT

Background: Inflammatory bowel diseases (IBD) are characterized by an increased thrombotic risk of uncertain etiology. Endogenous thrombin potential (ETP), a parameter of the thrombin generation curve, represents a new tool in the evaluation of thrombotic and bleeding disorders.
Aims: To study ETP in IBD patients and to correlate the results with clinical and biochemical features.
Methods: Seventy-four IBD patients (37 ulcerative colitis and 37 Crohn's disease) and 74 sex- and age-matched healthy individuals. ETP was measured upon activation of coagulation with small amounts of tissue factor and phospholipids in the presence or absence of thrombomodulin; results were expressed as nM thrombin·minutes.
Results: Mean±SD ETP values were significantly higher in patients (1,499±454) than controls (1,261±385) ($p<0.001$) only when the test was performed in the presence of thrombomodulin. ETP evaluated as ratio (with/without thrombomodulin), taken as an index of hypercoagulability, was significantly higher in patients (0.69±0.14) than controls (0.62±0.18) ($p<0.006$). Patients with increased C-reactive protein (CRP) had significantly higher mean ETP (1,721±458) than those with normal CRP (1,357±394) or controls (1,261±385) ($p<0.001$). Patients who at the time of blood sampling were classified as having a clinically active disease had ETP higher than those who were quiescent (1,655±451 versus 1,388±427, $p<0.001$) or controls (1,261±385, $p<0.001$).
Conclusions: ETP measured in the presence of thrombomodulin or as ratio (with/without thrombomodulin) is increased in IBD patients, mainly in those with increased CRP or active disease. It may be considered as a candidate test for prospective studies aimed at assessing the risk of thrombosis in IBD patients.

Introduction

Crohn's disease and ulcerative colitis, the two major forms of Inflammatory Bowel Diseases (IBD), are chronic illnesses characterized by local and systemic inflammation as well as by an increased risk of thrombotic events [1]. Thrombosis complicating IBD course may affect both the venous [2,3] and the arterial [4] district; they usually occur during active phases of the disease [1] and represent a relevant cause of morbidity and mortality due to the relatively young age of the affected patients [5,6]. Despite awareness is rising and the medical therapy of IBD is largely improved, the thrombotic risk does not appear to decline [7].

Several prothrombotic alterations have been described in IBD patients, such as quantitative and qualitative impairments of platelets [8], procoagulant [9] or fibrinolytic proteins [10] as well as hyperhomocysteinemia [11] or decreased naturally-occurring anticoagulant factors [12]. However, the exact pathogenic mechanisms of the thrombotic events are so far not fully elucidated.

Thrombin generation represents the final step of the coagulation cascade. It is triggered by the tissue factor in complex with activated factor VII and down-regulated by the naturally occurring anticoagulants [13]. In physiological conditions the balance between the procoagulant and anticoagulant drivers is essential to prevent excessive thrombin formation. Protein C and antithrombin are the two main anticoagulant proteins; the first is activated by thrombin in complex with the endothelial receptor thrombomodulin [14], the second mainly upon interaction with the glycosoaminoglycans located on endothelial cells [15].

The conventional coagulation tests such as the prothrombin and activated partial thromboplastin times (PT and APTT) do not contain

Abbreviations: ETP, Endogenous thrombin potential; PT, prothrombin time; APTT, activated partial thromboplastin time; CRP, C-reactive protein.

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sufficient amounts of thrombomodulin or glycosaminoglycans and are, therefore, unable to represent the balance of coagulation as it occurs in vivo [16]. In contrast, the last generation of assays that monitor the tissue factor induced thrombin generation in the presence of thrombomodulin are credited as better laboratory tools to represent the balance of pro- and anti-coagulant forces operating in plasma [16–18].

To the best of our knowledge, only one study assessed so far thrombin generation in a small group of patients with active ulcerative colitis [19] and the test was performed in the absence of thrombomodulin. This study aims to evaluate thrombin generation in the absence or presence of thrombomodulin in ulcerative colitis and Crohn's disease patients and to correlate the results with their biochemical and clinical features, with particular attention to the levels of the acute phase reactant C-reactive protein (CRP) and clinical disease activity. A more precise elucidation of the pathogenetic mechanisms underlying the thrombotic complications would be of value to identify patients at higher risk.

Materials and Methods

The Ethical Committee of our Institutions approved the study. All enrolled subjects were of Italian descent and gave their informed consent to participate in the study. Criteria of exclusion from the study were the previous history of thrombosis, anticoagulant treatment and the presence of cirrhosis or chronic pathologic conditions such as diabetes mellitus or renal failure.

Patients

We enrolled 74 consecutive IBD patients (42 men and 32 women, mean age ± SD: 44.7 ± 14.0 years) followed at our outpatient gastrointestinal clinics; 37 had ulcerative colitis and 37 Crohn's disease. The diagnosis of IBD was made by means of clinical, endoscopic, radiological, and histological findings. The clinical history of IBD patients was obtained from their clinical records. Disease activity was assessed by means of Crohn's disease activity index (CDAI) [20] and Truelove and Witts criteria [21] for ulcerative colitis; the clinical type of Crohn's disease was classified according to the Vienna classification [22]. The demographic and clinical features of IBD patients are summarized in Table 1.

Table 1
Demographic and clinical features of IBD patients.

	Crohn's disease	Ulcerative colitis
	n = 37	n = 37
Men/women	19/18	23/14
Mean age at sampling ± SD (*years*)	40.4 ± 12.3	48.9 ± 14.3
Mean disease duration ± SD (*months*)	70.0 ± 55.9	69.3 ± 55.8
Active disease activity at sampling, *n (%)*	11 (29.7)	20 (54.1)
CRP ≥ 0.5 mg/dl, *n (%)*	13 (35.1)	16 (43.2)
Location/extension, *n (%)*		
ileum	8 (21.6)	–
ileum+colon	17 (45.9)	–
colon	12 (32.4)	–
pancolitis	–	9 (24.3)
left colitis	–	16 (43.2)
procto-sigmoiditis	–	12 (32.4)
Behavior, *n (%)*		
non-stricturing, non-penetrating	11 (29.7)	–
penetrating	10 (27.0)	–
stricturing	16 (43.2)	–
Treatment, *n (%)*		
no therapy	5 (13.5)	2 (5.4)
only 5-ASA	17 (46.0)	25 (67.6)
steroids ± other drugs	15 (40.5)	10 (27.0)

Healthy subjects

As a control population we enrolled 74 sex- and age-matched healthy subjects (mean age ± SD: 43.4 ± 13.7 years) randomly picked up from a larger cohort of healthy individuals enrolled as controls for the studies of thrombophilia and thrombin generation in other clinical conditions carried out in the same period as the study in IBD patients. Their characteristics have been previously described [23]. Briefly, they were free from present and past thrombotic events, their prevalences of prothrombotic mutations were those observed in the general Italian population and carriers were not excluded from the study. None of the control subjects was on oral anticoagulants or other medications (including oral contraceptives) known to interfere with blood coagulation.

Blood collection and plasma preparation

Blood was obtained by clean venipuncture and collected in vacuum tubes containing 109 mmol/L trisodium citrate as an anticoagulant (Vacutainer; Becton Dickinson, Meylan, France) at a blood to anticoagulant ratio of 9:1. Blood was centrifuged within 30 minutes at (controlled) room temperature for 15 minutes at 2,880×*g*. The platelet-free plasma was subsequently aliquoted in plastic-capped tubes, quickly frozen in liquid nitrogen, and stored at -70 °C.

Methods

Coagulation parameters

Protein C was measured with a commercially available anticoagulant assays (PC Clot, Instrumentation Laboratory, Orangeburg, NY, USA). Factors VIII and II were measured as clotting activities by modifications of the APTT or the PT, respectively, and factor VIII or factor II deficient plasmas. Results for protein C, factors VIII and II were expressed as percentage activity relatively to a pooled normal plasma set arbitrarily at 100% activity.

C-reactive protein (CRP)

CRP was measured by a standard laboratory method and results were considered as increased when ≥0.5 mg/dL.

Thrombin Generation

Thrombin generation was evaluated according to Hemker et al. [24] as described in detail by Chantarangkul et al. [25]. The test is based on the activation of coagulation in test plasmas after addition of human relipidated recombinant tissue factor (Recombiplastin; Instrumentation Laboratory, Orangeburg, NY, USA) which acts as a coagulation trigger in the presence of the synthetic phospholipids 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS), 1,2-dioleoyl-sn-glycero-3-phosphoetanolamine (DOPE) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) (Avanti Polar Lipids Inc., Alabaster, AL) in the proportion of 20/20/60 (M/M). The concentrations of tissue factor and phospholipids in the test system were 1 pM and 1.0 μM, respectively. The experiments were also carried out by adding soluble rabbit thrombomodulin (ICN Biomedicals, Aurora, OH, USA) in the reaction mixture at a final concentration of 4 nM. This concentration was arbitrarily chosen upon experiments that showed the best discrimination of thrombin generated upon triggering coagulation for healthy subjects and patients with congenital protein C deficiency. Continuous registration of the generated thrombin was obtained by means of a fluorogenic synthetic substrate (Z-Gly-Gly-Arg-AMC HCl, Bachem, Bubendorf, Switzerland) added to the test system at a final concentration of 417 μM. The testing procedure was performed with an automated fluorometer (Fluoroskan Ascent; ThermoLabsystem, Helsinki, Finland) that is able to handle simultaneously several samples in an automated fashion. Readings from the fluorometer are recorded and calculated by a dedicated software (Thrombinoscope™, Thrombinoscope BV, Maastricht,