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Increased responsiveness to thrombin through protease-activated receptors (PAR)-1 and -4 in active Crohn's disease

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Abstract

Background and aims: Platelets are essential in hemostasis and inflammation, thereby linking coagulation with inflammation. Abundant thrombin generation in association with inflammation is considered a major reason for the increased risk for thromboembolic events. We therefore investigated platelet responsiveness to thrombin.
Methods: In this case–control study 85 patients with Crohn's disease (active CD 42, remission 43) and 30 sex- and age-matched controls were enrolled. Clinical disease activity (Harvey–Bradshaw-Index) was assessed and CD-related data were determined by chart review. Platelets' response to protease activated receptor-1 and -4 (PAR-1, -4) was assessed by whole blood platelet aggregometry (MEA), levels of platelets adhering to monocytes (PMA), and platelet surface P-selectin.
Results: Platelets' aggregation after activation with the specific PAR-1 agonist (SFLLRN) and PAR-4 agonist (AYPGKF) was higher in patients with active CD compared to patients in remission and controls ($p = 0.0068$ and $p = 0.0023$ for SFLLRN, $p = 0.0019$ and 0.0003 for AYPGKF). Likewise, levels of PMA after activation with PAR-1 and PAR-4 receptor agonists were higher in patients with active CD compared to patients in remission and controls ($p = 0.0001$ and $p < 0.0001$ for SFLLRN, $p = 0.0329$ and $p = 0.0125$ for AYPGKF). However, P-selectin expression on human platelets showed heterogeneous results. Only PAR-1 activation of platelets resulted in significant differences between CD patients and controls ($p = 0.0001$ and $p = 0.0022$ for active and inactive CD versus controls, respectively).

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Conclusions: Our data suggest a new mechanism of platelet activation which has the potential to increase risk for thromboembolism in patients with active CD which might be due to platelets poised for thrombin-inducible activation.
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1. Introduction

The two major forms of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), result from complex interactions between evolving environmental changes, more than 150[1] of predisposing genetic mutations, a complex gut microbiota that may be continuously varied, the intricacies of individual immune systems[2] and non-immune systems such as the hemostatic system.[3] Among these, platelets are believed to play a crucial role as it is evident that, in addition to their role in primary hemostasis, platelets also play an active role in inflammatory processes.[4,5] The concept of enhanced platelet activity in IBD is supported byreports showing significantly increased platelet aggregation in response to agonists like ADP, collagen, and ristocetin[6–8] in both forms of IBD.[9–11] While platelets are traditionally seen as the major players under high shear conditions like in atherosclerosis, there is growing evidence for their active role in venous thromboembolic disease.[12] We propose that the clinical importance of increased platelet activation is reflected by a substantially increased incidence of thromboembolisms in IBD.[13–18]

Continuous thrombin generation is considered a major reason for an increased risk of thromboembolic events, as it is a very potent platelet activator. Thrombin activates platelets via 4 distinct receptors, protease-activated receptors (PAR)-1, PAR-4,[19,20] glycoprotein (GP) Ibα and GPV.[21–24] PAR1 mediates platelet responses at low concentrations of thrombin while PAR4 mediates platelet activation only at high thrombin concentrations.[19] PAR-1 and PAR-4 form a heterodimeric complex[20,25] and with GPIbα complementary units a trimeric receptor complex regulating platelet activation by thrombin, that enables thrombin to act as a bivalent or even trivalent functional agonist. Thus, coordinate activation of PAR by subnanomolar thrombin concentrations has been proposed.[26] Clinically, there is strong evidence that PAR mediated platelet activation is significant.[27] IBD is associated with altered plasma levels of a variety of hemostatic biomarkers indicating subclinical activation of the coagulation system, finally leading to enhanced generation of thrombin.[28]

The complex interplay between inflammation and hemostasis results in activated platelets, aggregate formation, and release of P-selectin. The latter is the most important platelet "releasate" for the interaction with peripheral blood leukocytes, including monocytes, leading to enhanced leukocyte activation. High levels of platelet–monocyte aggregates (PMA) are regarded a very sensitive marker of thromboembolic disease.[29] Indeed, increased levels of PMA have been reported in a variety of inflammatory diseases[29–32] that are associated with an increased risk for thromboembolic disease, including IBD.[33,34]

We assessed the association between inflammation in CD patients with their platelets' responsiveness to thrombin. We compared the responsiveness of platelets to two major thrombin receptors, PAR-1 and −4, to their specific agonists (SFLLRN and AYPGKF for selectively activating PAR-1 and PAR-4, respectively) by platelet aggregation, determination of the formation of PMA, and levels of P-selectin expression of patients with active CD, patients in remission and controls. Thereby we show an enhanced responsiveness specific for PAR-inducible platelet activation in patients with active CD compared to patients in remission and controls. These findings may have the potential to explain the increased susceptibility for thrombin mediated risk of thromboembolic disease in patients with active CD.

2. Materials and methods

2.1. Patients and controls

The study complied with the Declaration of Helsinki, was approved by the Ethics Committee of the Medical University of Vienna, and all patients and healthy controls gave written informed consent. We enrolled 42 consecutive patients with active CD, 43 patients with CD in remission, and 30 age and sex-matched controls with coeliac disease under gluten-free diet without any symptoms as controls (Table 1). All participants were outpatients and recruited from the Department of Internal III, Division of Gastroenterology and Hepatology, Medical University of Vienna, Vienna, Austria. All CD patients were older than 18 years and had an established diagnosis of CD (based on clinical, endoscopic, histological, and radiological criteria according to European Crohn's and Colitis Organisation (ECCO) guidelines).[35] CD-related data were collected from chart review and included age at diagnosis, disease extent and behavior, CD-related surgery, medication, smoking habits, and disease activity. Extent of CD and disease behavior were classified according to the Montreal classification.[36] Active disease was defined by a Harvey–Bradshaw Index (HBI) of >4.[37] CD-related surgery was defined as bowel resection only. A smoker was defined as a patient who smoked at least 7 cigarettes weekly for at least 1 year.[38] The diagnosis of coeliac disease was based on typical morphology of duodenal mucosal biopsy specimens taken during oesophago-gastroduodenoscopy confirmed by positive antibodies, according to ESPGHAN criteria.[39] Patients with coeliac disease in long-term remission served as controls. They were symptom-free on gluten-free diet for at least one year and were anti-endomysial antibodies (EMA) and anti-tissue-transglutaminase antibodies — negative at any time of the study. Exclusion criteria included hereditary platelet abnormalities, a family or personal history of bleeding disorders, significant renal dysfunction, malignant paraproteinemias, myeloproliferative disorders, severe hepatic failure, malignancies, patients with adipose phenotype (BMI > 30 kg/m^2), a major surgical procedure within 3 months

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