Activations of Coagulation and Fibrinolysis Secondary to Bowel Inflammation in Patients with Ulcerative Colitis

Keiichiro Kume, Masahiro Yamasaki, Mitsuo Tashiro, Ichiro Yoshikawa and Makoto Otsuki

Abstract

Background Recent investigations suggest that activation of coagulation and fibrinolysis occurs in patients with ulcerative colitis (UC). However, the role of the hypercoagulable state in UC has not been determined. On the other hand, there are no reports dealing with coagulation in ischemic colitis (IC), in which acute bowel inflammation and reversible vascular occlusion affect the colon. Thus, our aim was to evaluate the hyper states of coagulation and fibrinolysis in UC by comparing activations of coagulation and fibrinolysis in patients with active UC and in those with IC.

Methods Twenty-four patients with active UC and 12 patients with IC were studied, with 18 patients with inactive UC serving as controls. We investigated the activation of the coagulation system, including platelet counts, activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT), serum concentrations of von Willebrand factor (vWF), activated factors XII, XI, X, IX, VIII, VII, V, II, fibrinogen, prothrombin fragments 1+2 (F1+2), thrombin-antithrombin complexes (TAT), protein S, protein C, plasminogen, α-2 plasminogen inhibitor (α-2PI) and D-dimer (D-D).

Results Median serum vWF concentrations, F1+2, TAT, fibrinogen, activated factor XI, IX, VIII and V were significantly elevated in patients with active UC and IC compared to those in patients with inactive UC. There was no significant difference between active UC and IC patients in the mean values of any of the factors that were measured.

Conclusion The results of the present study indicate that the coagulation-fibrinolysis system is activated in patients with active bowel inflammation such as active UC and IC, and that the hyper states of coagulation and fibrinolysis in patients with active UC are secondary to bowel inflammation.

Key words: ulcerative colitis, the hypercoagulable state

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Introduction

In patients with inflammatory bowel disease (IBD), mucosal and systemic hypercoagulation has been documented by several investigators (1-6). The increased risk of thromboembolic complications in patients with ulcerative colitis (UC) is well recognized (1-3). Based on these observations, heparin was used to treat or prevent thromboembolic events in UC patients (7-11). However, the efficacy of anticoagulant therapy, such as heparin, in UC patients remains controversial (7-12). Normalization of markers of the coagulation system has been documented during treatment, which suggests that the observed abnormalities are secondary to the disease state (5, 13). There have been no complete studies on the hypercoagulable state in UC on the total coagulation system, although there have been many reports on a part of the system (1-6, 13-32). Therefore, the first aim of this study was to compare active with inactive UC in relation to the hypercoagulable tendency in the total coagulation system.

Reversible vascular occlusion that affects the colon is called ischemic colitis (IC). The primary cause of the condition of IC is impaired perfusion, while the inflammatory reaction is secondary (33, 34). In most patients with IC, the disease requires no specific therapy such as anticoagulant therapy. However, there are no reports concerning the coagulation state in patients with IC. Thus, the second aim of
this study was to evaluate activations of coagulation and fibrinolysis in patients with IC, and to compare the hypercoagulation-fibrinolysis tendency among active, inactive UC patients and active IC patients.

**Patients and Methods**

**Patients**

This study included 54 patients: 24 with active UC, 12 with active IC, and 18 with inactive UC (controls) (Table 1). The median age was 37.6 (range 15-83) years. UC and IC were diagnosed based on the conventional clinical, radiological, endoscopic, and histological criteria. Disease activity of UC was assessed using a variation of the Truelove-Witts criteria (36). Patients with a score above 5 were regarded as having active colitis. Patients with active IC of less than 48 hours from onset were included. Patients with severe hepatic or renal dysfunction, proteinuria, malnutrition, or with a history of oral contraceptive use were excluded. No participants were on steroid and anticoagulant therapies at the time of the study.

**Laboratory studies**

Blood Sampling: Blood samples were taken from an antecubital vein puncture with minimal venous stasis, with the patient at rest in the morning. Blood was collected in two different plastic tubes, one containing sodium ethylenediaminetetraacetic acid (EDTA) for platelet counts and the other 3.8% sodium citrate for coagulation factors except platelet counts. Immediately after collection, platelet-free plasma was obtained by centrifugation at 3,000 rpm for 20 minutes at 4°C. The plasma was then snap frozen in aliquots and stored at -40°C until assayed.

Coagulation Measurements: Platelet counts, activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT), and serum concentration of fibrinogen (Fib) were measured using conventional methods. The serum concentrations of activated factors II, V, VII, X, VIII, IX, XI, and XII (IIa, Va, VIIa, Xa, XIa and XIIa) were assayed in stages using a parallel line bioassay based on APTT, TT, or PT. Plasma concentrations of thrombin-antithrombin complexes (TAT) and prothrombin fragments F1+2 (F1+2) were assessed using the enzyme-linked immunosorbent assay (ELISA) technique. Plasma levels of d-dimer (D-D) were determined using ELISA according to the manufacturer’s instructions. Antithrombin III (AT III) activity was measured using the chromogenic substrate S-2238. Serum concentrations of protein C activity were quantified using the chromogenic substrate S-2366. Functional protein S, α2-plasminogen inhibitor (α2-PI), and plasminogen levels in serum were determined using a commercial assay. Serum concentrations of von Willebrand factor (vWF) were determined using ELISA with commercially available von Willebrand factor antisera.

**Statistical Analysis:** Results are expressed as mean ± SD. The Kruskal-Wallis test and the Mann-Whitney U test with the Bonferroni correction were used for statistical analysis. A probability of less than 0.05 was considered to represent a significant difference between the samples studied. Data were analyzed using StatView (version 5, USA).

Informed consent was obtained from all patients, and the study protocol conformed to the ethical guidelines of the 1989 Declaration of Helsinki.

**Results**

The mechanism of hemostasis consists of two steps: thrombus formation and coagulation. The first step of thrombus formation occurs as a result of vascular injury and aids in the initiation of the coagulation system.

1. **The first step of thrombus formation (Table 2)**

The first step of thrombus formation inclusive of platelet count, vWF and fibrinogen were shown Table 2. Platelet counts in active UC patients were significantly higher than in inactive UC patients. The mean serum vWF level was significantly elevated in active UC and IC patients.
Table 2. Plasma Levels of the Parameters about the First Step of Thrombus Formation

<table>
<thead>
<tr>
<th>Parameter (normal range)</th>
<th>a-UC (n=24)</th>
<th>IC (n=12)</th>
<th>i-UC (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPTT (14.7-36.5) s x10^3 /μl</td>
<td>34.3±13.0*</td>
<td>27.4±6.6</td>
<td>25.4±5.8</td>
</tr>
<tr>
<td>vWF (60-170) %</td>
<td>165.8±56.2*</td>
<td>181.7±54.6*</td>
<td>109.1±11</td>
</tr>
</tbody>
</table>

a-UC: active ulcerative colitis, IC: ischemic colitis, i-UC: inactive ulcerative colitis

Results shown were mean ± SD

*: significant difference vs i-UC (p<0.05)

There was no significant difference between active UC and IC patients in the mean levels of any of the factors that were measured.

2. The coagulation system (Table 3)

The coagulation system consists of five steps.

1) The extrinsic pathway

There was no significant difference in PT among active UC, IC and inactive UC patients. The factor VIIa level in active UC and IC patients was higher than that in inactive UC patients.

2) The intrinsic pathway

There was no significant difference in APTT among the three groups of patients. The factor XIIa level in active UC and IC patients was higher than that in inactive UC patients. The mean level of factor XIa was significantly higher in active UC and IC patients than that in inactive UC patients.

3) The common pathway

There was no significant difference in TT among the three groups of patients. Factor IXa, VIIIa and Va levels were significantly elevated in active UC and IC patients compared to those in inactive UC patients. Factor Xa and AT-III levels in active UC patients were significantly higher than those in inactive UC and IC patients.

4) The thrombin-generating system

Factor IIa and protein C levels in active UC patients were significantly higher than those in IC and inactive UC patients. There was no significant difference in protein S level among the three groups of patients.

5) The fibrinolysis system

Fibrinogen, TAT, F1+2 and D-D levels were significantly elevated in active UC and IC patients compared to those in inactive UC patients. The α-2PI level in active UC patients was significantly higher than that in inactive UC patients, but was similar to that in IC patients. There was no significant difference in plasminogen level among the three groups of patients. There was no significant difference in the various coagulation parameters in between active UC and IC patients (Tables 2, 3).

Discussion

In the present study we found that the parameters of the total coagulation-fibrinolysis system were higher in active UC and IC patients than in inactive UC patients, and that the parameters of the total coagulation-fibrinolysis system in inactive UC patients were all within the normal range.

The incidences of thromboembolic events in patients with IBD have been reported to be 1%-8% (35-40). Patients with IBD have a 3-fold increased risk for deep vein thrombosis (DVT) and pulmonary embolism (PE) compared with the general population (37, 41). In addition, IBD patients experience thromboembolic events at younger ages than the general population (42). In contrast, patients with rheumatoid arthritis (inflammatory control) and patients with celiac disease (gastroenterologic disease) have fewer episodes of thromboembolism than IBD patients, even after adjusting for confounding variables such as operations, oral contraceptive pill use, smoking status and body mass index (37).

Disease activity is a probable risk factor for thromboembolism (40, 43). It is noteworthy however that one-third of thromboembolic complications occur in patients with quiescent disease (40). Therefore, we studied the hypercoagulability in active UC patients and compared it to that in inactive UC patients and IC patients with reversible ischemia requiring no specific therapies.

The hypercoagulable state in active UC patients is closely
Table 3. Plasma Levels of the Coagulation Parameters

<table>
<thead>
<tr>
<th>Parameter (normal range)</th>
<th>a-UC (n=24)</th>
<th>IC (n=12)</th>
<th>i-UC (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) The extrinsic pathway</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT (10.7-12.7) sec</td>
<td>11.1±1.0</td>
<td>11.3±0.8</td>
<td>11.4±0.7</td>
</tr>
<tr>
<td>VIIa (75-140) %</td>
<td>110.4±24.2</td>
<td>128.2±44.8*</td>
<td>96.6±17.6</td>
</tr>
<tr>
<td>2) The intrinsic pathway</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APTT (24.9-33.2) sec</td>
<td>29.5±5.1</td>
<td>28.3±3.8</td>
<td>31.1±4.2</td>
</tr>
<tr>
<td>XIIa (50-150) %</td>
<td>87.6±33.7</td>
<td>104.4±40.4*</td>
<td>69.1±23.0</td>
</tr>
<tr>
<td>XIa (75-145) %</td>
<td>106.3±24.9*</td>
<td>123.3±43.9*</td>
<td>78.5±17.3</td>
</tr>
<tr>
<td>3) The common pathway</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (60-100) %</td>
<td>87.8±30.3</td>
<td>85.9±19.6</td>
<td>96.8±27.2</td>
</tr>
<tr>
<td>IXa (70-130) %</td>
<td>122.1±26.0*</td>
<td>121.4±10.1*</td>
<td>91.8±16.1</td>
</tr>
<tr>
<td>Xa (70-130) %</td>
<td>112.5±20.7*</td>
<td>101.5±20.0</td>
<td>92.7±19.8</td>
</tr>
<tr>
<td>AT-III (91-127) %</td>
<td>116.1±22.1*</td>
<td>104.7±13.6</td>
<td>100.0±18.1</td>
</tr>
<tr>
<td>VIIIa (50-155) %</td>
<td>208.8±98.6*</td>
<td>200.0±22.4*</td>
<td>113.6±39.0</td>
</tr>
<tr>
<td>Va (70-135) %</td>
<td>118.0±23.2*</td>
<td>117.3±33.9*</td>
<td>88.0±11.1</td>
</tr>
<tr>
<td>4) The thrombin-generating system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIa (75-135) %</td>
<td>113.2±16.2*</td>
<td>110.8±24.3</td>
<td>94.4±16.9</td>
</tr>
<tr>
<td>Fib (200-400) mg/dl</td>
<td>363.5±85.8*</td>
<td>425.8±181.8*</td>
<td>254.9±126.3</td>
</tr>
<tr>
<td>TAT (&lt;3.0) ng/dl</td>
<td>4.1±1.9*</td>
<td>4.7±3.1*</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td>F1+2 (0.3-0.7) nmol/l</td>
<td>1.0±0.2*</td>
<td>1.0±0.2*</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>Protein S (60-150) %</td>
<td>95.5±20.3</td>
<td>96.1±34.7</td>
<td>83.9±18.0</td>
</tr>
<tr>
<td>Protein C (70-150) %</td>
<td>120.3±34.3*</td>
<td>109.5±27.2</td>
<td>96.4±18.3</td>
</tr>
<tr>
<td>5) The fibrinolysis system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasminogen (82-120) %</td>
<td>102.8±15.4</td>
<td>102.9±25.4</td>
<td>99.2±19.0</td>
</tr>
<tr>
<td>α 2-PI (87-119) %</td>
<td>113.4±12.0*</td>
<td>106.4±9.6</td>
<td>104.2±16.0</td>
</tr>
<tr>
<td>D-D (&lt;0.5) μg/ml</td>
<td>1.8±1.0*</td>
<td>2.4±2.3*</td>
<td>0.6±0.4</td>
</tr>
</tbody>
</table>

a-UC: active ulcerative colitis, IC: ischemic colitis, i-UC: inactive ulcerative colitis


Results shown were mean ± SD.

*: significant difference vs i-UC (p<0.05).

There was no significant difference between active UC and IC patients.
related to abnormal platelet function resulting in a high probability of microvascular thrombosis and microcirculatory dysfunction (14-16). On the other hand, vWF is a large glycoprotein that circulates in human plasma and is deposited in the vascular subendothelium. In response to endothelial injury, vWF also mediates platelet to platelet interactions and platelet adhesion to the subendothelium (44). We found that the median serum vWF concentration was significantly higher in active UC and IC patients than in inactive UC patients. The elevated vWF value in UC patients could be caused by either vascular injury occurring secondary to bowel inflammation or an acute phase response to endothelial cell stimulation by mediators released during the inflammatory process (17-19).

The coagulation system can be divided into extrinsic and intrinsic pathways (1). The intrinsic pathway is initiated when blood is exposed to a negatively charged surface, which activates factor XII (45, 46). Activation of factors XIIa and XIa have been reported in <10% of patients with UC (20, 21). We found that factor XIIa and XIa levels in active UC and IC patients were higher than those in inactive UC patients, and we considered that bowel inflammation might expose a negatively charged subluminal surface of inflamed vessels to peripheral blood. The extrinsic pathway is initiated by tissue damage that exposes the tissue factor (TF)-factor VII complex. It is thought that high factor VIIa activity is associated with an increased risk of ischemic myocardial events in man subjects over 40 years of age (18, 47). Hudson et al reported that the mean factor VIIa level was significantly higher in UC patients than in normal subjects (3). We also found that the factor VIIa level in active UC and IC patients was higher than that in inactive UC patients, and considered that increased concentrations of factor VIIa might complicate microvascular damage and inflammation in the intestinal wall by augmenting the focal fibrin deposition on the luminal surface of inflamed vessels.

Several investigators have reported that factor IXa, Xa, Va and VIIIa levels were elevated, while the AT-III level was decreased in active UC (2, 20-25). Our results revealed that factor IXa, Xa, Va, VIIIa and AT-III levels were higher in active UC and IC than inactive UC, and suggested that common pathways were initiated by extrinsic and intrinsic pathways.

Deficiencies of AT-III, protein C and protein S are well-recognized causes of thrombotic disease in UC patients (2, 17, 26). On the other hand, the present results suggested that there were no cases of hereditary thrombophilia in our study, since AT-III, protein C and protein S were within the normal range among all patients. It is known that the factor V Leiden mutation that contributes to resistance to activated protein C is rare in the Japanese population (48, 49).

F1+2 and TAT have been found to be reliable markers of thrombin generation. The present data also showed that mean F1+2 and TAT were significantly higher in active UC and IC than inactive UC, and suggested that thrombin generation might be an early event in UC and IC. It is well known that F1+2 and TAT are useful markers of the activation of blood coagulation in IBD (4-6, 27-30). Moreover, the activation of F1+2 and TAT is a risk factor for DVT and PE in IBD (48).

D-D is a marker of hyperfibrinolysis, and high D-D levels have been reported in IBD (4, 31, 32). Our results revealed that D-D was significantly higher in active UC and IC than inactive UC, and suggested the presence of intravascular thrombus in active UC and IC. We found that plasminogen levels were higher in active UC and IC patients than in inactive UC patients, whereas α2-PI showed no such difference. In was considered that the competition between lipoprotein(a) [Lp(a)] and plasminogen for cellular binding sites might contribute to the thrombotic and atherosclerotic risks associated with elevated Lp(a) levels (50).

Imbalances in hemostasis may be documented by measuring zymogens (Xa and prothrombin), cofactors (Va), the principle substrate (fibrinogen), and natural anticoagulants (AT-III and activated protein C). Because of an excess in plasma and because only a small fraction is activated and consumed during a prothrombotic state, these variables may be insensitive measures of coagulation activation (51). Activation peptides released from the zymogen molecules when activated may better document ongoing coagulation. The longer half-lives of these peptides compared with the activated serine protease is a further advantage (51). It is assumed that a balance exists between the activation of coagulation and fibrinolysis during normal physiologic circumstances (52). Thus, disturbances in the mechanisms of coagulation will presumably be reflected in the products of both coagulation and fibrinolysis (5).

The present active UC and IC patients showed similar results for the total coagulation-fibrinolysis system, and also they recovered without steroid or anticoagulant therapy. The parameters of the total coagulation-fibrinolysis system, except PT, APTT and TT, were higher in active UC and IC patients than in inactive UC patients, although the difference did not always reach statistical significance. The interaction between thrombosis and inflammation is becoming increasingly recognized (1-3, 36-43). Given this, the interest in the role of thrombosis in inflammatory conditions, including IBD, has increased. However, patients with IBD do not always require anticoagulant therapy. Our study showed that the presence of the hypercoagulation-fibrinolysis state was induced secondary to bowel inflammation, since the coagulation-fibrinolysis system was activated in active UC and IC patients compared to inactive UC patients.

It has been reported that the coagulation-fibrinolysis system is activated in not only UC but also Crohn’s disease (CD) (1-6). vWF, AT-III, F1+2, TAT, D-D, fibrinogen, VIII and V were activated in patients with CD similar to UC (13, 14, 17, 21-23, 25-27, 30-32, 37-43). Moreover, the activation of F1+2 and TAT is a risk factor for DVT and PE in CD similar to UC (48). Using histopathological analysis, Wakefield et al (53) identified a significant relationship between thrombus formation and angitis of the intestinal wall.
due to microthrombosis in the initial lesions of CD. Gaffney et al (10) reported fibrin thrombi were noted in the microvasculature of the colon in UC. Considered together, these results suggest that thrombi may be an important etiological factor in IBD. Some of the clinical events are peculiar in UC and are not observed in CD, such as bleeding from ulcerative lesions and bloody stools. These differences suggest heightened coagulative-fibrinolytic activities in UC (54).

It is widely accepted that IBD promotes thrombosis (1-6). It is important to deal with thrombotic complications when treating IBD patients. To date, anti-thrombotic compounds have not been shown to be therapeutically beneficial in IBD patients. Whereas, in the future, novel agents that target the involved pathways might add to the range of medications currently available. However, most importantly, studies are needed to clarify which IBD patients are at high risk of thromboembolic complications.

In conclusion, the hyper states of coagulation and fibrinolysis in active UC patients are secondary to bowel inflammation, since activation of coagulation and fibrinolysis were found not only in active UC but also in IC patients with active bowel inflammation.

References