Diagnostic Value of the Serum Platelet-Activating Factor Acetylhydrolase Activity in Inflammatory Bowel Disease

HIROKAZU OSHIMOTO, SHINICHI OKAMURA, TOMOHIRO IIDA, TAKESHI ISHIKAWA, KOHEI HOSAKA[1] and MASATOMO MORI

First Department of Internal Medicine, Gunma University School of Medicine, and [1] Department of Basic Sciences for Medicine, Gunma University School of Health Sciences, Maebashi, Japan

OSHIMOTO, H., OKAMURA, S., IIDA, T., ISHIKAWA, T., HOSAKA, K. and MORI, M. *Diagnostic Value of the Serum Platelet-Activating Factor Acetylhydrolase Activity in Inflammatory Bowel Disease.* Tohoku J. Exp. Med., 2005, **207** (1), 65-71 —— Platelet-activating factor acetylhydrolase (PAF-AH) is an enzyme hydrolyzing platelet-activating factor (PAF), a potent inflammatory mediator, but the relationship between this enzyme and inflammatory bowel disease (IBD) is not fully elucidated. The aim of the present study was to examine the usefulness of the serum PAF-AH activity in order to differentiate ulcerative colitis (UC) from Crohn's disease (CD). The serum PAF-AH activity was measured in 57 patients with IBD (39 UC and 18 CD patients) and 13 control subjects by a spectrophotometric method. The serum PAF-AH activity was thus found to be significantly lower in patients with CD (median 265.5 U/l) than in those with UC (355 U/l) or control subjects (374 U/l). This marker at a cutoff level of 386 U/l demonstrated a sensitivity of 46%, a specificity of 100%, and a positive predictive value of 100% regarding its ability to distinguish UC from CD. Moreover, the marker responded inversely to the changes in the disease activity of IBD. These results suggest that measuring the serum PAF-AH activity is a useful diagnostic modality for making a differential diagnosis between UC and CD. ———— platelet-activating factor acetylhydrolase; inflammatory bowel disease; ulcerative colitis; Crohn's disease
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Platelet-activating factor (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine [PAF]) is a potent inflammatory mediator that induces microvascular leakage, vasodilatation, smooth muscle contraction, and both platelet and leukocyte aggregation (Hanahan 1986; Snyder 1995). Increased amounts of PAF have been demonstrated in the colonic mucosa of patients with ulcerative colitis (UC) (Eliakim et al. 1988) and Crohn's disease (CD) (Sobhani et al. 1992), as well as in the stool of patients with infectious diarrhea (Denizot et al. 1992), pouch ileoanal anastomosis, and pouchitis (Chaussade et al. 1991). PAF is hydrolyzed by acetylhydrolase (PAF-AH [E.C.3.1.1.47]) into the biologically inactive lyso-PAF by removing the acetyl group at the sn-2 po-

sition of the glycerol backbone.

The PAF-AH activity has been demonstrated in a variety of biological materials including plasma (Nakamura et al. 1987; Miwa et al. 1988), colonic mucosa (Appleyard and Hillier 1992), and cultured intestinal epithelial cells (Kald et al. 1992). The PAF-AH activity in the plasma, which does not significantly differ from that in the serum (Kosaka et al. 2000), varies in such pathological conditions as hyperlipidemia, cardiovascular diseases, stroke, rheumatoid arthritis, sepsis, and asthma. This activity has also been observed to decrease in patients with neonatal necrotizing enterocolitis (Caplan et al. 1990). Regarding inflammatory bowel disease (IBD), this activity has been found to decrease in CD patients in comparison to controls in Sweden (Kald et al. 1996), but the level showed no difference between UC patients and controls in Japan (Nakamura et al. 2002). However, up to now, no direct comparison of the PAF-AH activity between UC and CD patients in the same population has yet been reported.

The aim of the present study was to determine the serum PAF-AH activity in Japanese IBD patients and to examine the usefulness of this measurement for differentiating UC from CD.

Subjects and Methods

Subjects

This study was performed on three groups of subjects: namely, a group of 39 UC patients, a group of 18 CD patients, and a group of 13 control subjects. In each individual of IBD, the diagnosis was based on the clinical, radiological, endoscopic, and histological findings. The control subjects consisted of patients with other miscellaneous colitides and diarrheal illnesses: five with irritable bowel syndrome, three with acute self-limited colitis, two with intestinal Behçet's disease, one with diverticulitis, one with eosinophilic enteritis, and one with amyloidosis. From the 16 IBD patients (9 UC, 7 CD), paired samples at two time points of serum and plasma were collected to determine the serum PAF-AH activity and the platelet count. The sera from five healthy volunteers were also collected. All serum samples were frozen and stored at -20°C until analysis.

The measurement of the disease activity included the International Organization of the study of Inflammatory Bowel Disease (IOIBD) assessment score for CD and clinical activity index (CAI) for UC. All pertinent clinical data were obtained from the hospital records. Informed consent was obtained from all subjects, in accordance with the Helsinki Declaration of the World Medical Association.

Measurement of PAF-AH activity

The measurement of the PAF-AH activity was carried out by a spectrophotometric method, as previously described by Kosaka et al. (2000), using an Azwell Auto PAF-AH kit (Alfresa Pharma Co., Osaka) according to the manufacturer's instructions. PAF-AH hydrolysis of the PAF analogues with 4-nitrophenyl groups was spectrophotometrically observed at 405 nm by monitoring the absorption change between 2 and 5 min after the addition of the substrate solution due to the liberation of 4-nitrophenol from the substrate through phospholipid hydrolysis.

Statistics

The positive and the negative predictive values are considered to depend on the prevalence of the disease in the general population. As a result, we used the prevalences per a population of 100,000 individuals in Japan of 18.12 for UC and 5.85 for CD (Morita et al. 1995).

Any statistical differences between two groups were determined by the Mann-Whitney's U-test. Statistical differences among the three groups were calculated using Steel-Dwass test after the Kruskal-Wallis test. The correlation between the serum PAF-AH activity and the platelet count was assessed by Spearman's rank correlation test and the correlation within subjects by multiple regression analysis (Bland and Altman 1995). A difference with a value of $p < 0.05$ was considered to be significant.

Results

Subject characteristics

The 3 groups had no significant differences regarding both age and gender. The characteristics of patients with IBD are summarized in Table 1. In UC, 49% of the patients had total colitis, 41% had left-sided colitis, and 10% had proctitis. In CD, 66% of the patients had both ileal and colonic involvement, 17% had only ileal involvement, and 17% had only colonic involvement. Salazosulfapyridine or mesalazine were prescribed to 90% of UC and 100% of CD.

TABLE 1. *Characteristics of patients with inflammatory bowel disease*

	UC	CD
Number of patients	39	18
M/F	19/20	10/8
Age (yr), median (range)	36 (20 – 74)	28 (16 – 46)
Disease extent		
Total colitis	19 (49%)	-
Left-sided colitis	16 (41%)	-
Proctitis	4 (10%)	-
Colon	-	3 (17%)
Ileum	-	3 (17%)
Ileocolonic	-	12 (66%)
Medication		
Salazosulfapyridine or mesalazine	35 (90%)	18 (100%)
Corticosteroids	10 (26%)	5 (28%)
Immunosuppressants	1 (3%)	2 (11%)
Infliximab	-	0

Corticosteroids and immunosuppressants were respectively administered to 26% and 3% of UC, and 28% and 11% of CD.

Serum PAF-AH activity

The serum PAF-AH activity was found to be significantly lower in patients with CD (median 265.5 U/l) than in those with UC (355 U/l; $p < 0.05$) or control subjects with miscellaneous colit-
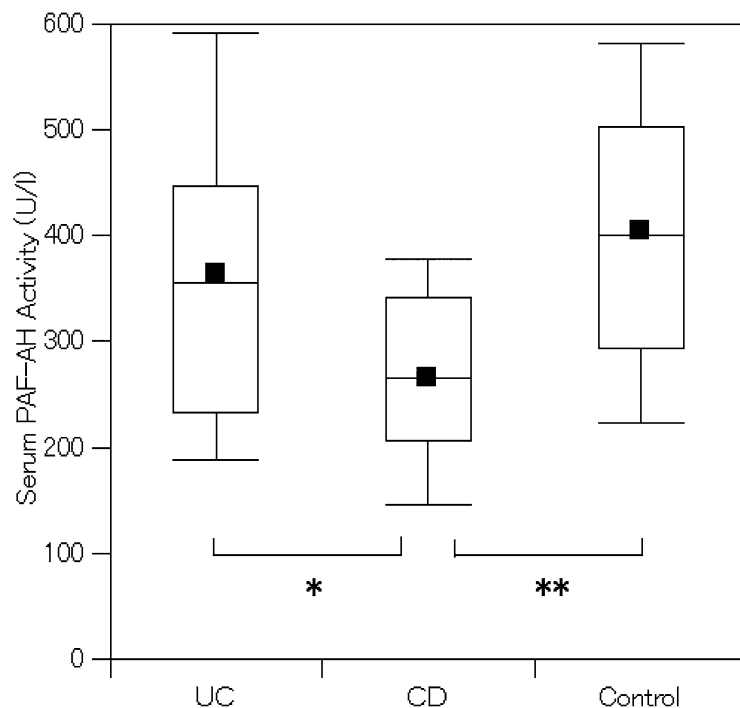

Fig. 1. The serum PAF-AH activity in IBD and controls.

The serum PAF-AH activity in ulcerative colitis ($n = 39$), Crohn's disease ($n = 18$), and controls ($n = 13$). The control subjects consisted of patients with other miscellaneous colitides and diarrheal illnesses: five with irritable bowel syndrome, three with acute self-limited colitis, two with intestinal Behçet's disease, one with diverticulitis, one with eosinophilic enteritis, and one with amyloidosis. Bars represent the 10th, 25th, 50th (median), 75th, and 90th percentiles of each group from the bottom, respectively. Hatched boxes show the mean of each group. $^{*}p < 0.05$, $^{**}p < 0.01$ (Steel-Dwass test after the Kruskal-Wallis test).

ides and diarrheal illnesses (374 U/l; $p < 0.01$) (Fig. 1). The median value of the activity in healthy volunteers ($n = 5$) was 427 U/l. This activity was observed to be significantly higher in proctitis (644.5 U/l) in comparison to either left-sided colitis (350 U/l; $p < 0.05$) or total colitis (339 U/l; $p < 0.05$). After the exception of proctitis, UC (346 U/l) still had a significantly higher activity than CD (265.5 U/l; $p < 0.05$).

In paired samples from the 16 IBD patients (9 UC, 7 CD), the PAF-AH activity showed an inverse response to the alterations in the disease ac-

TABLE 2. *Responses of the serum PAF-AH activity to changes in the disease activity of IBD*

	Serum PAF-AH activity	
	Increased	Decreased
Clinical activity		
Exacerbated	0	5 (4 UC, 1 CD)
Stable	3 (3 CD)	5 (4 UC, 1 CD)
Ameliorated	3 (1 UC, 2 CD)	0

The disease activity and the serum PAF-AH activity were determined in patients with IBD (9 UC and 7 CD) at two time points and their changes were summarized. The disease activity, the clinical activity index (CAI) for UC and the International Organization of the study of Inflammatory Bowel Disease (IOIBD) assessment score for CD.
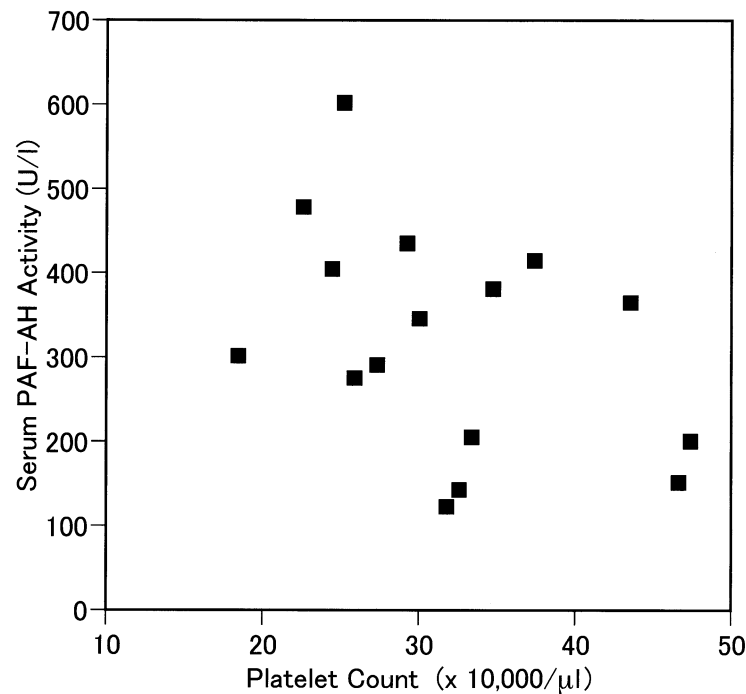

Fig. 2. The serum PAF-AH activity and platelet count in IBD.
The serum PAF-AH activity and platelet count were determined in patients with IBD (9 UC, 7 CD) at two time points and their means were plotted. Spearman's rank correlation coefficient was – 0.42 ($p = 0.103$).

TABLE 3. *Predictive power of the serum PAF-AH activity to distinguish ulcerative colitis from Crohn's disease*

	UC (n = 39)	CD (n = 18)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PAF-AH (+)	18	0	46	100	100*	37*
PAF-AH (−)	21	18				

PPV, positive predictive value; NPV, negative predictive value. PAF-AH (+), the serum PAF-AH activity ≧ 386 U/l. * These values were determined using the prevalences per a population of 100,000 individuals in Japan of 18.12 for UC and 5.85 for CD (Morita et al. 1991).

tivity of IBD (Table 2), but no correlation was noted between the level of PAF-AH activity and the extent of the disease activity. The correlation between the serum PAF-AH activity and the treatment with corticosteroids was not significant. In addition, this marker inversely correlated with the platelet count (Spearman's rank correlation coefficient, –0.42; $p = 0.103$) (Fig. 2) and this correlation within subjects was found to be significant by multiple regression analysis (correlation coefficient, –0.87; $p < 0.0001$).

Discriminating UC from CD based on the serum PAF-AH activity

To discriminate UC from CD based on the serum PAF-AH activity, the optimal cutoff level of this activity was determined to be 386 U/l by drawing a receiver operating characteristic (ROC) curve using different cutoff levels; analysis by the ROC produced an area under the curve of 0.708 (95% confidence interval 0.614 to 0.775). This cutoff level showed a sensitivity of 46% with a specificity of 100% and a positive predictive value of 100% (Table 3). The false positive rate and the false negative rate were calculated to be 0% and 54%, respectively. Next, the optimal cutoff level of the activity to distinguish CD from controls was determined to be 348 U/l with a sensitivity of 83%, a specificity of 77%, and a positive predictive value of 83%.

DISCUSSION

In the present study, we demonstrated that CD patients have a significantly decreased serum PAF-AH activity in comparison to UC patients

and found that the serum PAF-AH activity may potentially make it possible to differentiate UC and CD.

We showed that the serum PAF-AH activity had the sensitivity, specificity, positive predictive value, and negative predictive value of 46%, 100%, 100%, and 37%, respectively, in order to distinguish UC from CD. As a result, a high positive predictive value (100%) would thus allow an accurate discrimination in 46% of the UC patients. In contrast to this, two of the most commonly used serologic markers for distinguishing UC from CD are perinuclear anti-neutrophil cytoplasmic antibody (pANCA) and anti-saccharomyces cerevisiae antibody (ASCA), which either alone or in combination demonstrated sensitivity, specificity, positive predictive values and negative predictive values ranging from 30% to 67%, 84% to 98%, 54% to 96%, and 41% to 86%, respectively (Quinton et al. 1998; Koutroubakis et al. 2001; Peeters et al. 2001; Hisabe et al. 2003; Lawrance et al. 2004). Therefore, the serum PAF-AH activity may be a relatively more useful marker than ASCA and ANCA. The effect of combination use of the serum PAF-AH activity, ASCA, and ANCA deserves further investigation because the latter two have higher discriminating power in combination than either alone (Quinton et al. 1998; Koutroubakis et al. 2001; Peeters et al. 2001).

The present study did not target at indeterminate colitis. The diagnosis remains unclear in from 5 to 15 percent of IBD patients (Peeters et al. 2001; Guindi and Riddell 2004), and such cases are temporarily classified as having indeterminate

colitis, and such a distinction may be particularly difficult in patients with fulminant colitis (Swan et al. 1998). The study focusing on the discrimination between UC and CD in indeterminate colitis by measuring the serum PAF-AH activity should be conducted in the near future because there are fundamental differences in disease management and prognoses between UC and CD.

Our study disclosed that UC had a significantly increased level of serum PAF-AH activity in comparison to CD and this activity in UC was significantly higher in proctitis than in other types of UC. Although the mechanism of these findings is open to further investigation, one possible explanation is that these differences may be caused by the leakage of serum PAF-AH protein into the intestinal lumen through the affected intestine in accordance with the disease extent or location (Denizot et al. 1992; Guimbaud et al. 1995; Kald et al. 1996).

The present study showed an inverse alteration of the serum PAF-AH activity in response to the changes in the disease activity of IBD and a negative correlation between the PAF-AH activity and the platelet count, which has been reported to positively correlate with the disease activity (Nielsen et al. 2000). These findings suggest that the serum PAF-AH activity is a potential marker for the disease activity of IBD. To date, an inverse relation between the plasma PAF-AH activity and the disease activity has been demonstrated in CD patients (Kald et al. 1996). More recently, UC patients with lower plasma PAF-AH activity due to a PAF-AH gene mutation have been found to be refractory to steroid therapy and to be operated with higher rate (Nakamura et al. 2002). Further research is called for to clarify the relationship between the serum PAF-AH activity and the disease activity of IBD.

One of the benefits of measuring the serum PAF-AH activity in IBD is its simplicity and non-invasiveness. Although the discrimination and clinical follow-up of IBD usually need radiography, endoscopy, and histopathology (Sanders 1998; Tanaka et al. 1999), these examinations are often intolerable for some patients such as children and the elderly. The measurement of the se-

rum PAF-AH activity may reduce the burden and increase the compliance of the patients.

In conclusion, measuring the serum PAF-AH activity is a potentially useful diagnostic modality for clinically differentiating UC from CD. Further investigations could increase the clinical usefulness of this marker.

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