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• CLINICAL RESEARCH •

Plasma matrix metalloproteinase-1 and tissue inhibitor of metalloproteinases-1 as biomarkers of ulcerative colitis activity

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Abstract

AIM: Overexpression of mucosal metalloproteinases (MMP) have been demonstrated recently in inflammatory bowel disease. Their activity can be counterbalanced by the tissue inhibitor of metalloproteinases (TIMP). The aim of this study was to evaluate the effect of ulcerative colitis (UC) on MMP-1 and TIMP-1 plasma concentrations, as two possible biomarkers of the disease activity.

METHODS: MMP-1 and TIMP-1 plasma concentrations were measured with an enzyme immunoassay in 16 patients with endoscopically confirmed active UC.

RESULTS: Plasma concentrations of both MMP-1 (13.7±0.2 ng/ml) and TIMP-1 (799±140 ng/ml) were significantly elevated in UC patients in comparison to healthy controls (11.9±0.9 ng/ml and 220±7 ng/ml respectively). There was no correlation between TIMP-1 and MMP-1 concentrations (r=-0.02). TIMP-1 levels revealed significant positive correlations with scored endoscopic degree of mucosal injury, disease activity index and clinical activity index values as well as C-reactive protein concentration. There was no correlation between MMP-1 and laboratory, clinical or endoscopic indices of the disease activity.

CONCLUSION: These results confirm the role of both MMP-1 and TIMP-1 in the pathogenesis of ulcerative colitis. However only TIMP-1 can be useful as a biomarker of the disease activity, demonstrating association with clinical and endoscopic pictures.

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INTRODUCTION

Pathogenesis of ulcerative colitis (UC) is focused on abnormal immune response and diminished ability of mucosal protection and regeneration. These processes are controlled by signaling between epithelial cells involving complex network of cytokines, growth factors and other bioactive substances, responsible for cell proliferation and differentiation, as well as regulation of immune response[1-3]. Alterations in synthesis and breakdown of extracellular matrix components are known to play a crucial role in tissue remodeling during inflammation and wound healing. Inflammatory bowel disease (IBD) is sometimes complicated by stricture formation and muscle hypertrophy resulting from extracellular matrix (ECM) changes related to matrix metalloproteinases (MMPs) activity[4]. Overexpression of mucosal MMPs have been demonstrated recently in inflammatory bowel disease[5-7]. Effects of MMPs activity can be counterbalanced by the tissue inhibitor of metalloproteinases-1 (TIMP-1)[8]. The MMP/TIMP ratio imbalance plays an important role in many diseases including not only inflammatory bowel disease but also chronic liver injury[8] and carcinogenesis[9]. As we demonstrated recently, elevated plasma concentration of TGF-β_1, known as TIMP-1 stimulator, was related to inflammation activity and should be considered as a possible biomarker in UC patients[11,12]. According to Sch ppan and Hahn[10] blockade of certain MMPs could be a novel th rapeutic approach, and therefore some novel mucosa derived parameters, such as MMP-1 and TIMP-1, may prove useful to assess prognosis, disease activity, and treatment response in inflammatory bowel disease.

The aim of this study was to evaluate effect of ulcerative colitis on MMP-1 and TIMP-1 plasma concentrations, as two possible biomarkers of the disease activity.

MATERIALS AND METHODS

Patients

MMP-1 and TIMP-1 concentrations were measured in the plasma of 16 patients (6 females and 10 males) with active ulcerative colitis (UC), and age ranging from 25 to 68 years (mean: 42.5±3.8). All the patients had a history of diagnosed ulcerative colitis that required typical clinical and endoscopical signs of distal part of bowel involvement. MMP-1 and TIMP-1 plasma concentrations were compared with endoscopic picture scored according to Meyers *et al*.[13], the disease activity index (DAI) according to Schroeder *et al*.[14], clinical activity index (CAI) as previously described[11] and laboratory indices of UC activity such as C-reactive protein (CRP), sedimentation rate (SR), white blood count (WBC) and hemoglobin concentration. Plasma MMP-1 and TIMP-1 concentrations were also compared with those of 12 healthy controls (mean age: 40.8±2.7 years). The study was approved by the Bioethical Committee of the Medical University in Bialystok. Informed consent was obtained from each patient.

MMP-1 and TIMP-1 measurement

Venous blood was collected on ice using vacutainer tubes with EDTA as an anticoagulant and centrifuged at 1 000×g within 30 minutes of collection. Obtained plasma was additionally centrifuged at 10 000×g for 10 minutes at 2-8 ℃ for complete platelet removal and stored at -20 ℃. The samples were diluted 1:40 with 0.1M phosphate buffer before assay and then incubated in duplicate in microtitre wells precoated with anti-TIMP-1 or anti-MMP-1 antibodies (Amersham Pharmacia Biotech, Little Chalfont, Buckinghampshire, England). Any TIMP-1 or MMP-1 remained in the microtitre wells after four cycles of washing and aspiration were detected by peroxidase

labelled specific antibodies. The amount of peroxidase bound to each well was determined by the addition of tetramethylbenzidine substrate. The reaction was stopped by acidification and optical density was read with a microtitre plate photometer Stat Fax® 2100 (Alab/Poland) at 450 nm. The concentration of MMP-1 or TIMP-1 in the sample was determined by interpolation from a standard curve prepared with standard samples supplied by the manufacturer.

Statistical methods

Values were expressed by their mean and standard error of the mean (±SEM). The significance of the difference was calculated by two-tailed Student' s *t* test. Correlation analysis was calculated by the non-parametric Spearman rank order correlation test. Values of $P<0.05$ were considered to be significant.

RESULTS

Plasma mean concentration of MMP-1 measured in patients with active UC was significantly elevated (13.7±0.2 ng/ml) in comparison to that of healthy controls (11.9±0.9 ng/ml) (Figure 1). Mean TIMP-1 plasma concentration in UC patients (799±140 ng/ml) also exceeded normal values significantly (220±7 ng/ml), and the difference was much more apparent (Figure 2). Even the lowest TIMP-1 value (456 ng/ml) doubled the mean concentration from the controls. As demonstrated in Table 1, the majority of patients had CRP and SR values exceeding the upper limit of normal range. Moreover evaluation of the disease activity through CAI, DAI and endoscopic score demonstrated severe course of the present relapse (Table 1). There was no correlation between TIMP-1 and MMP-1 plasma concentrations (r=-0.02, P=0.95). As shown in Table 2 plasma TIMP-1 levels analyzed in UC patients revealed significant positive correlations with scored endoscopic degree of mucosal injury, DAI and CAI values as well as CRP concentration. There was no correlation between MMP-1 and laboratory, clinical or endoscopic indices of the disease activity (Table 2).

Table 1 Laboratory and clinical indices of ulcerative colitis activity in the patients

	Normal range	Mean	±SEM	Minimum	Maximum	Median
CRP (mg/dl)	0-5	17.7	4.6	6	62	6
SR (mm/h)	0-12	22.7	4.7	2	68	17
WBC (×10³/μl)	4-10	6.8	0.5	3.5	11.5	6.2
HGB (mg/dl)	12-16	13.3	0.3	10.5	14.9	13.4
CAI	0	10.6	0.75	7	15	9
DAI	0	6.4	0.7	3	10	5
Endoscopic score	0	11.6	0.8	8	16	10

CAI: clinical activity index, DAI: disease activity index.

Table 2 Correlation between plasma TIMP-1 or MMP-1 concentrations and values of selected laboratory indices, demonstrated through "r" values. Statistically significant correlation: [a]$P<0.05$; [b]$P<0.01$

	TIMP-1 (r)	MMP-1 (r)
CRP (mg/dl)	0.60[a]	0.05
SR (mm/h)	0.17	-0.07
WBC (x10³/ml)	0.24	-0.10
HGB (mg/dl)	-0.19	-0.10
CAI (clinical activity index)	0.55[a]	-0.18
DAI (disease activity index)	0.66[b]	-0.27
Score	0.66[b]	-0.11

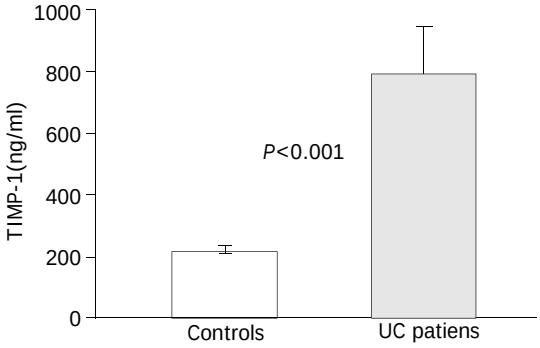

Figure 1 Comparison of mean (±SEM) TIMP-1 plasma concentrations in group of patients with ulcerative colitis and controls.

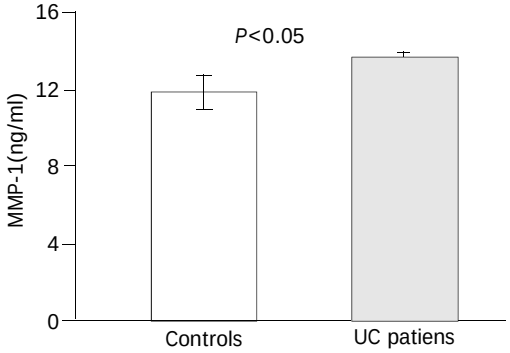

Figure 2 Comparison of mean (±SEM) MMP-1 plasma concentrations in group of patients with ulcerative colitis and controls.

DISCUSSION

Matrix metalloproteinases are involved in mucosal degradation causing tissue remodelling and ulcerations related to inflammatory bowel diseases. Enhanced expression of MMP-1, MMP-2 and MMP-3 has been demonstrated recently in inflammed mucosa from patients with UC and Crohn disease[5,6]. According to Arihiro *et al*.[15] in both UC and CD, MMP-1, MMP-9 and TIMP-1 were expressed by inflammatory cells, fibroblastic cells as well as by vascular smooth muscle cells most prominently in actively inflamed areas in ulcer bases. Stimulation of T lymphocytes in inflammatory lesions seemed to be responsible for activation of several MMPs[7,16,17]. TNF-α released by T lymphocytes was a powerful inducer of fibroblasts that were the prime source of MMPs[19,20]. This created link between mucosal inflammation and destruction of the subepithelial matrix, and MMP-1 expression in the mucosa might be related to the initial step of ulceration in UC[14]. According to Baugh *et al*.[20] the expression of matrix metalloproteinases 1, 2, 3 and 9 was significantly higher in inflamed areas of UC patients mucosa compared with noninvolved regions. In the study carried out by von Lampe *et al*.[5] MMP-1 and -3 correlated well with the histological degree of acute inflammation resulting in more than 15-fold increased levels in inflamed versus normal colon samples from patients with UC. In another study MMP-1 and -2 concentrations measured (using sandwich ELISA) in samples from pouchitis and active UC doubled the values obtained in samples of uninflamed mucosa. Mesenchymal cells were identified as major producers of MMP-1 and -2[6]. MMPs were implicated in the tissue destruction associated with inflammatory diseases and the role of MMPs in the pathogenesis of inflammatory bowel disease was also confirmed through improvement of experimentally induced colitis after treatment with a matrix metalloproteinase inhibitor[21].

Apart from protease-inhibitory action TIMP-1 serves

additional functions. Several investigators have demonstrated its growth-promoting properties and stimulation of tumor growth by inhibiting apoptosis indicating the role of TIMP-1 in cancer progression[22,23]. According to Holten-Andersen *et al*.[24] plasma TIMP-1 levels could be used to identify patients with colorectal cancer with a sensitivity of 63 % and a specificity of 98 %, so it was suggested as a marker for early identification of this cancer.

According to Heuschkel *et al*.[25] TIMP-1 measured in biopsies from patients with active IBD remained unchanged. In the study of Louis *et al*.[26] the production of TIMP-1 was undetectable in the majority of uninflamed biopsy samples from controls, UC and CD patients. However in inflamed mucosa, the production of TIMP-1 was increased significantly in both UC and CD. Its elevated plasma concentration, demonstrated in our study, can reflect situation in bowel mucosa. Enhanced expression of TIMP-1 can be a result of the stimulatory effect of transforming growth factor TGF-β_1. As we demonstrated recently, enhanced production of TGF-β_1 could be related to inflammation activity in UC patients[11]. This profibrogenic cytokine accelerated healing but during chronic inflammation might lead to excessive collagen deposition and eventually fibrosis[4]. In our recent study successful treatment of the disease resulted in decrease of its levels both in plasma and rectal mucosa, but better response has been achieved in patients with higher baseline TGF-β1 concentrations[12].

MMP-1 is the main enzyme responsible for degradation of fibrillar collagen and therefore we decided to use it as a possible biomarker in our study[6]. We demonstrated a significant increase of both MMP-1 and TIMP-1 plasma concentrations in UC patients, which could reflect their over-expression in the bowel mucosa. However significant correlation with clinical and endoscopical signs of UC activity was proved only for TIMP-1. Moreover the only laboratory parameter that showed any association with TIMP-1 was C-reactive protein.

In conclusion, our data confirm the role of both MMP-1 and TIMP-1 in the pathogenesis of ulcerative colitis. However only TIMP-1 may be useful as a biomarker of the disease activity, demonstrating association with clinical and endoscopic pictures.

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