

INVESTIGATIVE REPORT

Effect of Psoriasis Activity on Metalloproteinase-1 and Tissue Inhibitor of Metalloproteinase-1 in Plasma and Lesional Scales

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The aim of this study was to evaluate the association between psoriasis severity and concentrations of matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in plasma and scales from psoriatic lesions, measured with an enzyme immuno assay in 24 patients and analysed with respect to psoriasis area and severity index (PASI). The mean plasma concentrations of both proteins in psoriatic patients significantly exceeded the control values. The proteins were also detectable in scales. There was a significant correlation between plasma MMP-1 concentration and the disease duration. The PASI values showed significant positive correlation with plasma TIMP-1 and significant negative correlation with MMP-1 content in scales. The highest plasma MMP-1 concentration was observed in patients with mild forms whereas the highest plasma TIMP-1 concentrations were demonstrated in severe forms of psoriasis. Our results confirm the role of these proteins in pathogenesis of psoriasis. In severe forms, a decrease in both MMP-1 and TIMP-1 was observed in scales, suggesting their insufficient tissue expression, which can be a crucial element of psoriasis aggravation. *Key words: psoriasis; metalloproteinase-1; tissue inhibitor of metalloproteinases-1; psoriasis area and severity index (PASI); skin diseases.*

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Hyperproliferation and altered differentiation of keratinocytes associated with an inflammatory infiltrate in the epidermis is characteristic of psoriasis (1). The cause of hyperproliferation in psoriasis is still unknown, but it can be a result of the persistent autocrine stimulation of the epidermal growth factor receptor by transforming growth factor- α (TGF- α) and additional effect of interleukin (IL)-2, IL-6 and IL-8 released by T cells in the inflammatory infiltrates. The imbalance of factors responsible for epidermal proliferation is important for the pathogenesis of psoriasis and can be a possible target for therapeutic intervention (2–5). Epithelial cell proliferation can be inhibited by TGF- β_1 . Its effects on growth

and differentiation have been extensively characterized in cultured keratinocytes and can be considered important for the pathogenesis of psoriasis (6–9). This effect can be mediated at least in part by the Mad1 transcription factor, as demonstrated recently by Werner et al. (10). TGF- β_1 , which is predominantly produced in the upper differentiated layers of the epidermis, has been found to antagonize the effects of TGF- α considered to be responsible for hyperproliferation of psoriatic keratinocytes (3, 4, 11). As we have demonstrated recently, plasma levels of TGF- β_1 are strongly associated with psoriasis severity and decrease during efficient treatment but only in patients with the severe form of the disease (12, 13). TGF- β_1 is also known as a stimulator of tissue inhibitors of metalloproteinases (TIMP), affecting the activity of extracellular matrix metalloproteinases (MMPs). The possible role of TIMP and MMP in the pathogenesis of psoriasis has been demonstrated only in few studies, most of which showed enhanced expression of both MMPs and their inhibitors (14–19). However, according to Feliciani et al. (14) TIMP-1 and TIMP-2 are not expressed in psoriatic lesions in contrast to MMP.

It has been demonstrated that psoriasis-related keratinocyte hyperproliferation can be at least in part down-regulated by the TGF- β_1 -dependent TIMP-1/MMP-1 system (14–19). However, the results are not convergent and do not analyse the effect of psoriasis activity on these proteins. We undertook this study to evaluate the association between psoriasis severity and concentrations of TIMP-1 or MMP-1 in plasma and scales collected from psoriatic lesions, which can support the hypothesis as to their possible role in the pathogenesis of psoriasis.

MATERIALS AND METHODS

Patients

Concentrations of TIMP-1 and MMP-1 were measured in plasma collected from 24 patients (6 women and 18 men, aged 14–69 years, mean 40.0 ± 2.8) with chronic plaque-type psoriasis. Patients with a history of any other inflammatory chronic disease and subjects with other forms of psoriasis were not included in the study. Concentrations of TIMP-1 and MMP-1 were measured before the beginning of treatment, so the study outcome was not affected by medication. The results were analysed with respect to the disease activity demonstrated through the psoriasis area and severity index (PASI). Patients

were divided into three groups with PASI values <15, 15–20 and >20. Analysis of TIMP-1 and MMP-1 levels was also performed with respect to sex, age, familial prevalence of the disease (confirmed psoriasis in first degree relative), effect of the sun on remission of lesions, disease duration (years from the first relapse to sample collection) and duration of present relapse (weeks from appearance of papular lesions to sample collection). TIMP-1 and MMP-1 values were also compared to sedimentation rate, platelet count and white blood count as laboratory signs of inflammation. Normal values of plasma TIMP-1 and MMP-1 were determined in a study of 12 healthy controls. Mean age of controls (47.0 ± 2.0 years) did not differ significantly ($p=0.11$) from the group of patients. The study was approved by the Bioethical Committee of the Medical University of Białystok, and informed consent was obtained from each patient.

PASI

PASI was calculated according to the rules proposed by Fredriksson & Pettersson (20). The head, trunk, upper and lower limbs were assessed separately for erythema, infiltration and desquamation. The degree of severity of each symptom in each body part was scored from 0 to 4. The area covered by lesions of a particular body part was assigned a score from 0 to 6. The score for each of the four body parts was obtained by multiplying the sum of the severity scores of the three symptoms by the area score, then multiplied by the constant weighted value assigned to a particular body part as follows: head 0.1, trunk 0.3, upper limbs 0.2, lower limbs 0.4. The sum of the scores of body parts gives the PASI. PASI was evaluated by one investigator (I.F.) in all patients.

MMP-1 and TIMP-1 measurement

Venous blood was collected on ice using vacutainer tubes with EDTA as an anticoagulant and centrifuged at 1000 g within 30 min of collection. Plasma samples were additionally centrifuged at 10000 g for 10 min at $2-8^{\circ}\text{C}$ for complete removal of platelets. Scales were collected on ice from plaque lesions demonstrating the most intensive inflammation and desquamation, mashed immediately with homogenizer in buffer (50 mM Tris-HCl, 75 mM NaCl, 1 mM phenylmethyl sulphonyl fluoride) and centrifuged at 1000 g within 30 min of collection. Plasma and scales samples were stored at -20°C for up to 7 days. Thawed samples were diluted 1:40 with 0.1 M phosphate buffer before assay and incubated in duplicate in microtitre wells precoated with anti-TIMP-1 or anti-MMP-1 antibodies according to the procedure recommended by the manufacturer (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK). 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 ($\pm$ 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

The mean plasma concentrations of TIMP-1 and MMP-1 in psoriatic patients were significantly higher ($p=0.012$ and $p=0.042$, respectively) than control values (Table I). Both TIMP-1 and MMP-1 were detectable in scales. A comparison performed between concentrations of TIMP-1 and MMP-1 in plasma and scales did not reveal significant correlation. No correlation was found between TIMP-1 and MMP-1 in plasma, but there was a significant correlation between the concentrations of these peptides in scales. We found no significant differences with respect to sex and no significant correlation with age. Analysis of TIMP-1 and MMP-1 levels performed with respect to familial prevalence of the disease and beneficial effect of the sun on remission of lesions revealed no significance. As shown in Table II, there was a significant correlation between the disease duration and MMP-1 concentration in plasma but not in scales. The levels of both proteins did not demonstrate any association with the duration of present relapse. A significant correlation was also noted between plasma TIMP-1 and sedimentation rate but not with respect to platelets and white blood count. There were no correlations between plasma or scale MMP-1 concentrations and sedimentation rate.

PASI varied from 6 to 41.2 (mean: 16.4 ± 1.2) and was not related to age or sex. The analysis of PASI values

Table I. Tissue inhibitor of metalloproteinases-1 (TIMP-1) and metalloproteinase-1 (MMP-1) concentrations (mean $\pm$ SEM, and range) in plasma and scales of patients with psoriasis

	Source	Mean $\pm$ SEM	Range	<i>p</i> value*
TIMP-1	Plasma (ng/ml)	1523 ± 107	781–3131	0.012
	Normal value	1102 ± 67		
	Scales (ng/mg)	23.3 ± 2.7	2.1–53.6	–
MMP-1	Plasma (ng/ml)	24.3 ± 3.1	8.1–81.2	0.042
	Normal value	11.9 ± 0.9		
	Scales (ng/mg)	16.0 ± 3.2	0.9–73.2	–

**p* values reflect comparison to normal values (for plasma samples only).

Table II. Duration of the disease, sedimentation rate and psoriasis area and severity index (PASI) score and their correlations (*r* value) with plasma or scales tissue inhibitor of metalloproteinases-1 (TIMP-1) and metalloproteinase-1 (MMP-1) concentrations.

Parameter	Mean $\pm$ SEM	<i>r</i> value			
		TIMP-1		MMP-1	
		Plasma	Scales	Plasma	Scales
Disease duration (years)	18.2 ± 1.79	0.035	-0.209	0.391*	-0.121
Sedimentation rate (mm/h)	12.4 ± 2.0	0.413*	-0.307	-0.052	-0.207
PASI score	16.4 ± 1.2	0.507*	-0.089	-0.264	-0.357*

* $p < 0.05$.

showed a significant correlation with plasma TIMP-1 levels ($r=0.507$, $p<0.01$) (Table II, Fig. 1). Moreover, there was a significant negative correlation between PASI and MMP-1 scale content ($r=-0.357$, $p<0.05$) (Table II, Fig. 2). As demonstrated in Fig. 3, mean concentrations of TIMP-1 in plasma increased, whereas they decreased in scales, depending on the degree of psoriasis activity as measured by PASI score. Plasma TIMP-1 concentration was significantly higher than normal in patients with PASI values exceeding 15 (1609 ± 210 ng/ml, $p=0.022$) and 20 (1697 ± 162 ng/ml, $p=0.001$), respectively. Moreover, there was a statistically significant difference ($p=0.026$) between plasma TIMP-1 in groups with PASI<15 and >20. TIMP-1 concentration in scales demonstrated an opposite trend, but without significant differences between the groups (Fig. 3). As shown in

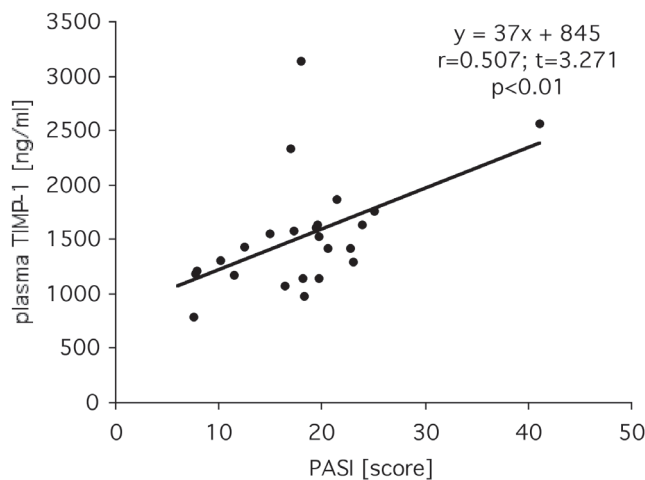


Fig. 1. Correlation between plasma tissue inhibitor of metalloproteinases-1 (TIMP-1) and psoriasis area and severity index (PASI) in patients with psoriasis.

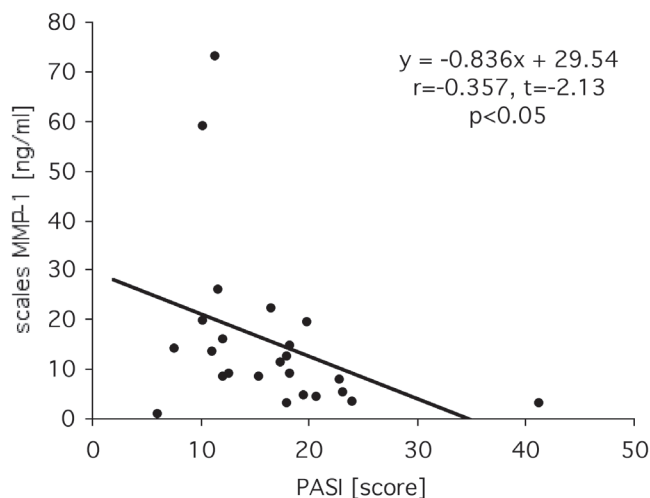


Fig. 2. Correlation between concentration of matrix metalloproteinase-1 (MMP-1) in scales and psoriasis area and severity index (PASI) in patients with psoriasis.

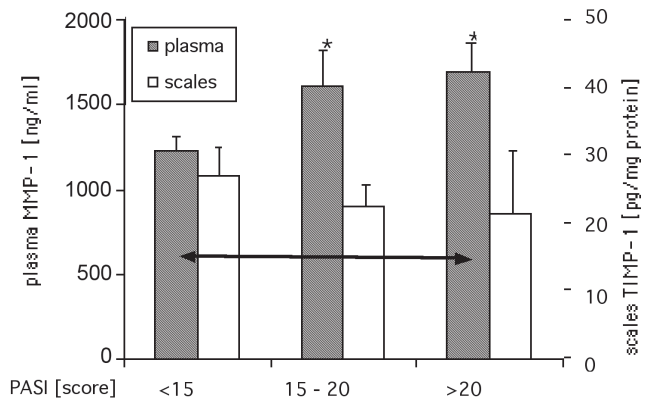


Fig. 3. Mean concentration of tissue inhibitor of metalloproteinases-1 (TIMP-1) in plasma and scales collected from psoriatic patients with respect to degree of psoriasis severity. Arrow indicates statistical significance ($p=0.026$) between groups; asterisks indicate statistical significance in comparison to controls (1102 ± 67 ng/ml; $p=0.022$ and 0.001 , respectively).

Fig. 4, the highest plasma MMP-1 mean concentration (27.2 ± 7.8 ng/ml) was observed in patients with a mild form of the disease (PASI<15). Values observed in this group as well as in patients with moderate activity of the disease (PASI 15–20; 18.4 ± 1.7 ng/ml) were significantly higher than in the control group ($p=0.035$ and $p=0.002$, respectively). The MMP-1 content of scales also demonstrated a decreasing tendency in patients with a more severe form of the disease; however, there were no statistically significant differences between the groups (Fig. 4).

DISCUSSION

Overexpression of gelatinases A and B (MMP-2 and MMP-9) in psoriatic skin was described for the first time in 1997 by Feliciani et al. (14). They found no TIMP-1 or TIMP-2 expression and concluded that overexpression of gelatinases without associated expression of the

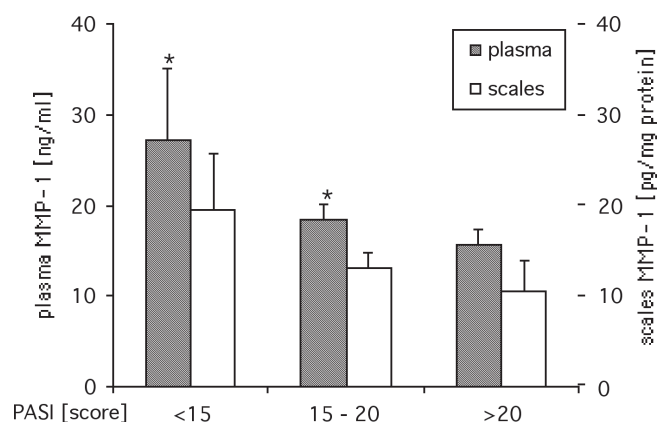


Fig. 4. Mean concentration of metalloproteinase-1 (MMP-1) in plasma and scales collected from psoriatic patients with respect to degree of psoriasis severity. Asterisks indicate statistical significance in comparison to controls (see text).

corresponding inhibitors suggests a role of these proteins in psoriatic skin remodelling. According to Buisson-Legendre et al. (15), the increased level of MMP-9 demonstrated in psoriatic keratinocyte cell culture might compete for TIMP-1 binding to its receptor. A study performed by Fleischmajer et al. (16) with cytochemistry, Western blot and *in situ* hybridization demonstrated overexpression of MMP-2 and TIMP-2 in uninvolved and involved psoriatic epidermis. This observation supported the concept that the primary alteration in psoriasis may reside in the keratinocytes. Enhanced MMP production causes destruction of extracellular matrix proteins and through damage of basement membrane favours angiogenesis and formation of inflammatory infiltrations, which together with hyperproliferation of keratinocytes, acanthosis and degeneration are a typical feature of psoriasis (17, 18). On the other hand, Suomela et al. (19) and Sadowski et al. (21) have demonstrated recently that up-regulation of MMP-19 in keratinocytes from psoriatic lesions can be a result of changes in the basement membrane zone. Previous research has demonstrated abundant expression of TIMP-1 in the inflammatory infiltrates as crucial for psoriasis (22).

We applied PASI for scored evaluation of the disease severity, which is generally an accepted method of standardization for psoriasis assessment. Alternative methods developed to improve quantitative evaluation of psoriasis activity, including computer image analysis, are not yet widely accepted (20). Since collection of skin samples using biopsy procedures for research purpose is ethically doubtful, we decided to perform measurements in scales.

Although the few studies on MMP and TIMP are not convergent and in part even contradictory, they clearly indicate a role of these proteins in the pathogenesis of psoriasis. All studies demonstrate overexpression of metalloproteinases, mostly MMP-2 and MMP-9 in psoriatic skin or psoriatic keratinocyte cell culture, but to date there has been no analysis of association between these enzymes and the disease activity. We showed elevated concentrations of MMP-1 in scales from psoriatic lesions, but interestingly enough its levels decreased in patients with a more severe form of the disease, which was confirmed through a significant negative correlation with PASI. A similar tendency was also observed with respect to concentrations of TIMP-1 in scales. In contrast, plasma concentrations of TIMP-1 showed a significant positive correlation with PASI. Thus, severe forms of psoriasis seem to be associated with a tissue deficit of TIMP-1/MMP-1 complex in superficial skin layers and the reason can be related to insufficient expression of specific receptors resulting in higher TIMP-1 plasma levels. On the other hand, a possible effect of MMP-1 and TIMP-1 degradation by other proteinases should also be considered as a cause of their decreased level in scales. The association observed between plasma

MMP-1 concentration and the disease duration seems not to be rational and statistical significance was lost when values at the extremes were deleted. As we performed measurements in psoriatic scales, it is difficult to link our results with MMP-related stimulation of angiogenesis (characteristic of psoriasis) and its possible inhibition by TIMP. Elevated concentrations of TIMP-1 and TIMP-2 in serum and synovial fluid in psoriatic arthritis and psoriasis without arthritis have also been demonstrated recently (23, 24). In our study, elevated plasma concentration of TIMP-1 was associated with decreasing concentrations of MMP-1. We are not able to answer the question why this effect was not observed in scales. However, this observation is consistent with the known inhibitory effect of TGF- β_1 on epithelial cells and correlation of its levels with the disease severity (12). As TGF- β_1 is an important inducer of TIMP-1, down-regulation of this system can be responsible for hyperproliferation related to the predominant effect of TGF- α (6–9).

In conclusion, our results demonstrated a strong association between plasma TIMP-1 concentrations and psoriasis severity, indicating the role of this protein in the pathogenesis of psoriasis. However, in severe forms of the disease a decrease in both MMP-1 and TIMP-1 was observed in scales, suggesting their insufficient tissue expression, which can be an important element of psoriasis aggravation.

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