Expression of metalloproteinases and their inhibitors in skin lesions of systemic sclerosis (SSc) patients

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Abstract

Metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases – TIMPs) are implicated in variations of extracellular matrix composition. Disregulation of ECM degradation is considered to play a key role in the pathogenesis of systemic sclerosis (SSc). Influence of MMPs and TIMPs on ECM composition may result not only from regulation of their production by fibroblasts, but also their direct role in the inflammatory process.

The aim of the present study was to investigate the expression of selected MMPs and their inhibitors in skin lesions of patients with SSc. MMP-1, MMP-2, MMP-9, MMP-10, TIMP-1, TIMP-2 and TIMP-3 were evaluated, by means of immunohistochemistry, in skin biopsies from 10 patients with SSc as well as 10 age-matched healthy controls.

Expression of MMPs and TIMPs was elevated in the skin of SSc patients in comparison with healthy skin. The overexpression of MMPs and TIMPs in SSc specimens is due to the presence of MMP-1, MMP-2, MMP-9, MMP-10, TIMP-2 and TIMP-3 within inflammatory infiltrate. In addition, certain MMPs (MMP-2 and MMP-9) and TIMP-2 were present in the stromal cells. Evaluation with three-step semiquantitative scale revealed that in SSc patients TIMPs expression appeared relatively lower when compared with MMPs.

The results of this preliminary study indicate that there are local disturbances in the expression of MMPs and their inhibitors in the skin of patients with SSc. It suggests that altered expression of MMPs/TIMPs may contribute to the development of local inflammatory infiltrated and tissue fibrosis in systemic sclerosis.

Key words: *metalloproteinases, tissue inhibitors of metolloproteinases, systemic sclerosis, immunohistochemistry.*

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Introduction

Pathogenesis of systemic sclerosis is complex and still not fully elucidated. It is probably associated with influence of many different, mutually cooperating factors [1].

The role of disturbances in composition of extracellular matrix (ECM), especially excessive accumulation of collagens type I, III, V and VI, fibronectin and tenascin, is postulated in development of fibrosis associated with systemic sclerosis [2]. It remains unexplained, however, whether such disturbances result from increased synthesis or decreased degradation of ECM components. It is suggested that surplus of these components may result from disproportion between degrading proteases and their inhibitors.

Metalloproteinases belong to the group of enzymes digesting the components of ECM. Their action depends

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on the presence of active zinc and calcium ions. MMPs are synthesized and secreted by various cells, such as fibroblasts, monocytes, granulocytes, T cells, Langerhans dendritic cells, macrophages, endothelial cells, epithelial cells, myocytes, neurons, astrocytes, as well as keratynocytes [3-5]. Literature data suggest that tissue inhibitors of proteolytic enzymes, produced by the same types of cells, may play a greater role in fibrosis process than the enzymes themselves [6]. Tissue inhibitors influence both the activation process of metalloproteinases and their already active forms [7]. So far tissue inhibitors of four metalloproteinases: MMP 1, 2, 3 and 4 were identified, and function of three of them was fully recognized [8]. Production of MMPs is regulated by cytokines, growth factors, cell-cell and cell-matrix interactions, as well as processes controlling gene expression and activation of their proenzymatic forms. The activity of MMPs is also modulated by their specific tissue inhibitors. Both matrix metalloproteinases and their tissue inhibitors have important role in the course of inflammatory processes [9, 10].

Imbalance between enzymes degrading ECM components and their inhibitors plays a role in pathogenesis of many diseases, including certain autoimmune dermatoses, such as pemphigoid and herpetiform dermatitis [11-23]. It was demonstrated that both soluble and cellular membrane-bound metalloproteinases have a role in degradation of the basement membrane and may be the cause of bullae formation [24, 25].

Disturbed homeostasis between MMPs and their inhibitors may be involved in changes of the extracellular matrix composition.

The aim of the present study was to investigate the expression of selected MMPs and their inhibitors in skin lesions of patients with SSc.

Material and methods

Patients

The studied group comprised 10 patients with systemic sclerosis (SSc), 6 with limited SSc (lSSc) and 4 with diffuse SSc (dSSc), 9 women and 1 man, mean age 54.4 years, range 38 to 72) (table 1). The disease was diagnosed on the base of American College of Rheumatology (ACR) criteria for systemic sclerosis [26].

Basic laboratory tests and the following additional examinations were performed in all patients: esophageal scintigraphy, 24-hour ECG monitoring, Doppler echo-cardiography, chest X-ray, lung spirometry, X-ray of feet and hand bones. Antinuclear antibodies serum levels (ANA) were identified by the indirect immunofluorescence and precise identification of antibodies was performed by the double immunodiffusion method (DID) according to Outchterlony [27].

Mean duration of Raynaud's phenomenon was 9.7 years, range 4 to 20, and duration of skin sclerosis was 5.4 years, range 1-16. Mean Total Skin Score was 15 points, range 10 to 28. Number of internal organs involved was from 1 to 3. Scl 70 antibodies were present in sera of 5/10 SSc patients, U3RNP in 1 patient with lSSc and in 4 another noncharacteristic antinuclear anibodies were found.

Patients with diffuse form of SSc were treated with low doses of prednisone (20 mg/day) and/or immunosuppressive drugs (cyclophosphamid 50 mg/day). All medication was continued during our study because of severe course of the disease and ethical concerns.

Vasodilatory drugs and vitamin E were administered for patients with lSSc.

Control group consisted of 10 healthy volunteers (5 women and 5 men), aged between 19 and 49 years (mean 42 years), without skin disease.

Before entering the study all the patients and controls gave written informed consent and the study was approved by Bioethics Committee of Medical University of Lodz.

Tissues

Punch biopsies were taken from indurated skin on dorsal aspect of the hands in SSc patients. The specimens were formalin-fixed and paraffin-embedded prior to examination. Punch biopsy specimens from the wrist region were also obtained from 10 healthy volunteers.

Immunohistochemistry

Paraffin-embedded sections (3-4 μm thick) were used for routine H+E staining and for immunohistochemistry in DAKO EnVision detection system using immunoperoxidase method. The following primary mouse monoclonal antibodies were used: anti-MMP 1, anti-MMP 2, anti-MMP 9,

Table 1. Clinical characteristics of examined group

Parameter	Statistical parameters	SSc (n=10)	Control group (n=10)
Sex (F/M)		9/1	5/5
Age (years)	mean	54.4	42
	range	38-72	19-49
Duration of Raynaud's phenomenon (years)	mean	9.7	–
	range	4-20	
Duration of skin sclerosis (years)	mean	5.4	–
	range	2-16	
TSS (points)	mean	15	–
	range	10-28	
Number of involved internal organs	mean	2	–
	range	1-3	
ANA		10	0

anti-MMP 10 and anti-TIMP 1, anti-TIMP 2 and anti-TIMP 3 (Novocastra). In order to avoid non-specific staining background blocking antibodies from control set (DAKO) were used. For immunohistochemistry, the paraffin-embedded sections were placed on adhesive plates and dried at 56°C for 24 hours, later deparaffinated in a series of xylens and alcohols of decreasing concentrations (96%, 80%, 70%, 60%). Activity of endogenous peroxidase was inhibited with 3% hydrogen peroxide solution in methanol for 5 minutes.

In order to retrieve the antigenicity of tissues and allow them to react with antibodies, specific procedures were used for each antibody, according to manufacturers' instruction. After incubation with diluted antibodies for 60 minutes at room temperature, slides were washed with TRIS buffer twice. Then, DAKO EnVision double-step visualization system was applied to visualize the antigen-antibody reaction. In cases of positive immunohistochemical reaction cellular nuclei were stained with Meyer's haematoxylin for 2 minutes. After dehydratation and processing through series of acetones and xylenes, as described above, the sections were fixed in Canadian balm.

The three-step semiquantitative scale was applied for evaluation of the intensity of immunohistochemical reaction with examined markers. The first step (weak intensity) – immunohistochemical reaction was limited to the single epithelial cells and/or was only focally present in the stroma. The second step (moderate intensity) – immunohistochemical reaction was observed in part of epithelial cells and/or was present in some areas of the stroma. The third step (strong intensity) – immunohistochemical reaction was observed in numerous epithelial cells and/or was present in large areas of the stroma.

Expression of MMPs and TIMPs was assessed by two independent pathologists using Nikon Microfob FXA microscope (Nikon LTD, Japan).

Table 2. Expression of examined metalloproteinases and their tissue inhibitors in skin biopsies

Localization	Keratinocytes	Stromal cells	Perivascular infiltrate	Parenchymal infiltrate	Hair follicles
Patients (n=10)					
MMP 1	3	nd	7	10	2
MMP 2	3	10	7	10	nd
MMP 9	5	3	8	10	nd
MMP 10	1	1	7	10	nd
TIMP 1	7	nd	1	10	nd
TIMP 2	9	nd	9	10	1
TIMP 3	8	nd	10	10	nd
Controls (n=10)					
MMP 1	sk	4	nd	nd	nd
MMP 2	sk	4	nd	nd	nd
MMP 9	sk	4	nd	nd	nd
MMP 10	sk	4	nd	nd	nd
TIMP 1	sk	4	nd	nd	nd
TIMP 2	sk	4	nd	nd	nd
TIMP 3	sk	4	nd	nd	nd

sk – single keratinocytes; nd – non detectable.

Results

In all patients histological examination (H + E) revealed changes typical for scleroderma.

Expression of examined metalloproteinases and their tissue inhibitors is presented in table 2.

Matrix metalloproteinases in SSc biopsy specimens

Collagenase (MMP-1)

Moderate expression of MMP-1 was found in 10 out of 10 specimens. In 7 biopsies expression of MMP-1 was detected in perivascular and parenchymal infiltrates (monocytes and lymphocytes) and in 2 also in hair follicles. In 3 out of 10 specimens it was localized in basal keratinocytes (figure 1).

Gelatinase (MMP-2)

A very intensive signal for gelatinase was detected in all samples. In all of them it was observed in stromal cells

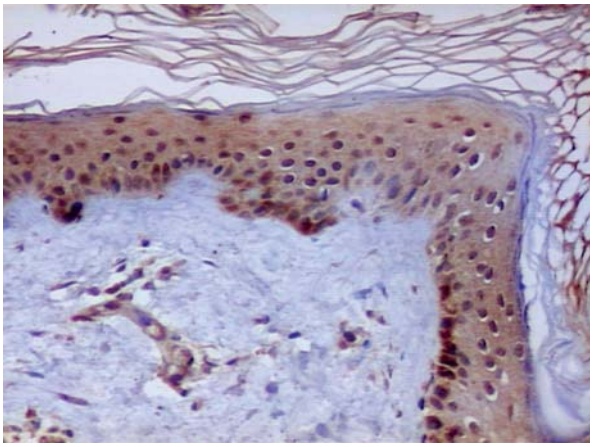

Fig. 1. Collagenase (MMP 1) (100x). Skin lesions. Immunohistochemistry. Moderate expression of collagenase (MMP 1) in basal keratinocytes and perivascular infiltrate (mag. 100x)

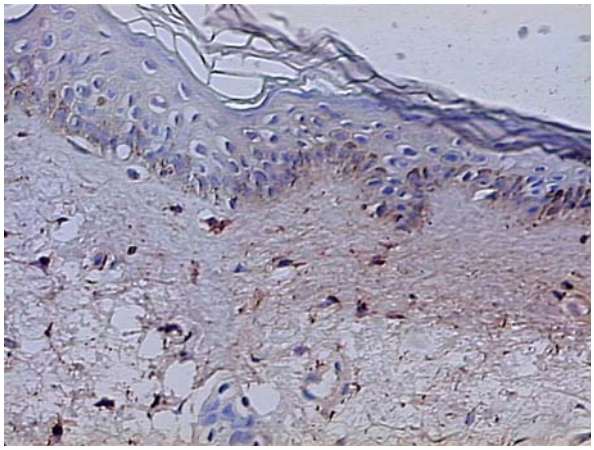

Fig. 2. Gelatinase (MMP 2) (100x). Skin lesions. Very intensive expression of gelatinase (MMP 2) in stromal cells and perivascular infiltrate (mag. 100x)

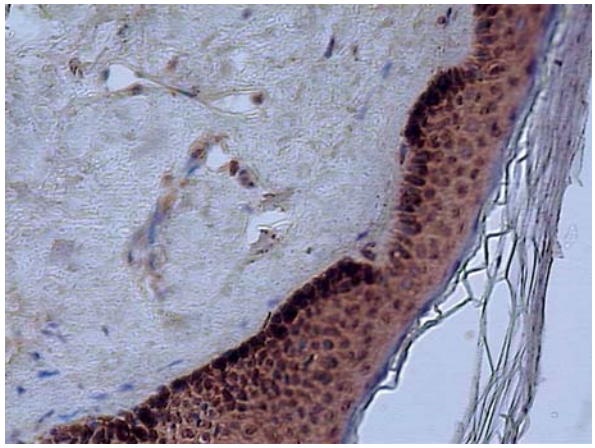

Fig. 3. 92 kD gelatinase (MMP 9) (100x). Skin lesions. Strong expression of 92 kD gelatinase (MMP 9) in basal keratinocytes (mag. 100x)

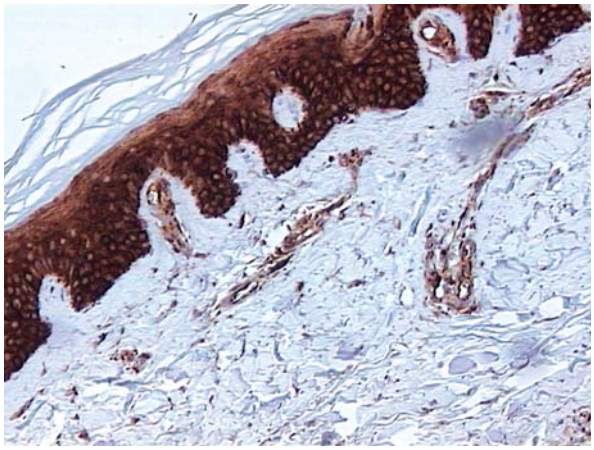

Fig. 4. Stromelysin (MMP 10) (100x). Skin lesions. Intensive expression of stromelysin (MMP 10) in whole epidermis and in perivascular infiltrates (mag. 100x)

(fibrocytes, fibroblasts, mastocytes) and in 7 out 10 specimens MMP-2 expression was also detected in perivascular infiltrates (figure 2). Gelatinase expression was present also in basal keratinocytes (3 out of 10).

92 kD gelatinase (MMP-9)

In all of 10 biopsies strong expression of MMP-9 was observed. In 8 out of 10 biopsies expression of this metalloproteinase was found in perivascular infiltrates, in 5 of them in basal keratinocytes (figure 3) and in 3 specimens it was present also in stromal cells. Only in 2 out of 10 biopsies expression of this metalloproteinase was observed only in basal keratinocytes. The most distinct expression of this gelatinase was found in parenchymal infiltrate in 1 out of 10 specimens.

Stromelysin (MMP-10)

High expression of MMP-10 was observed not only in perivascular infiltrates (7 out of 10) but also in basal keratinocytes and in the whole epidermis (1 out of 10) (figure 4). Only in 1 specimen it was also detected in stromal cells and parenchymal infiltrates of monocytes and lymphocytes.

Tissue inhibitors of metalloproteinases

In contrast to metalloproteinases expression of their tissue inhibitors was assessed as moderate or weak in all examined biopsies

TIMP 1

Expression of TIMP 1 was found not only in basal keratinocytes but also in cells of the whole epidermis (7 out of 10) (figure 5). Only in 1 out of 10 specimens positive signal for TIMP 1 was observed also in perivascular infiltrate. No expression of this tissue inhibitor was found in stromal cells.

TIMP 2

Moderate signal for TIMP 2 was observed in perivascular infiltrates and in the whole epidermis in 9 out of 10 specimens (figure 6). In 1 biopsy moderate positive staining for this tissue inhibitor was present in hair follicles.

TIMP 3

Weak or moderate expression of TIMP 3 was present in perivascular infiltrates in all of 10 specimens and also in the basal keratinocytes (8 out of 10) (figure 7) and only in 2 out of 10 specimens weak signal for TIMP 3 was observed in the whole epidermis.

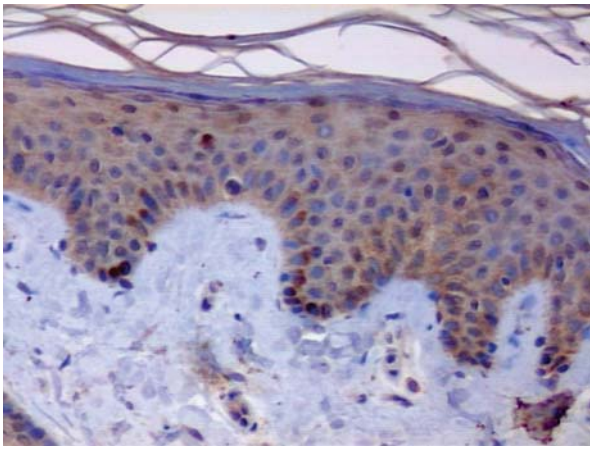

Fig. 5. TIMP 1 (400x). Skin lesions. Moderate expression of TIMP 1 in whole epidermis (mag. 400x)

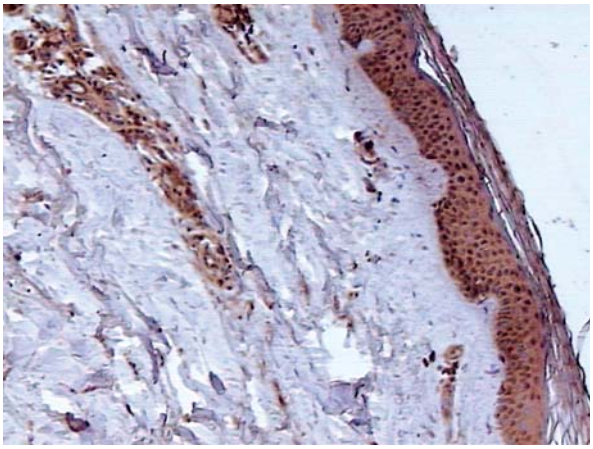

Fig. 6. TIMP 2 (100x). Skin lesions. Moderate expression of TIMP 2 in perivascular infiltrates and in the whole epidermis (mag. 100x)

Infiltrate

In parenchymal cellular infiltrate (monocytes and lymphocytes) expression of all the MMPs and TIMPs was observed. It was the most distinct for MMP 1, MMP 2 and MMP 10, as well as for TIMP 2. In stromal cells no expression of TIMP 1 and TIMP 3 was found. The most intense signal was noted for MMP 2 and MMP 10 (table 2).

Controls

Expression of MMP 1, MMP 2, MMP 9, MMP 10 and TIMP 1, TIMP 2, TIMP 3 was examined using the same method in 10 skin samples obtained from healthy volunteers. In all biopsies only few basal keratinocytes showed moderate expression of all the studied proteins (figure 8). In 4 biopsies weak positive staining for the above enzyme was present in hair follicles.

Discussion

Fibrosis process in the course of systemic sclerosis depends on many factors. It results not only from immune and vascular disturbances, but also from increased production and deposition of collagen and other ECM components in connective tissue. Results of numerous studies point to certain role of metalloproteinases and their inhibitors in this process [3, 28-30].

Enzymes degrading the components of ECM include: collagenases, gelatinases, elastases, stromelysins and membrane metalloproteinases [31]. It was demonstrated that the following metalloproteinases: MMP 1, MMP 8 and MMP 13 possess the ability to cleave fibers of type I, II and III collagens [30]. Gelatinases, MMP 2 and MMP 9, degrading denaturated collagen and cellular membrane components also significantly influence the composition of extracellular matrix

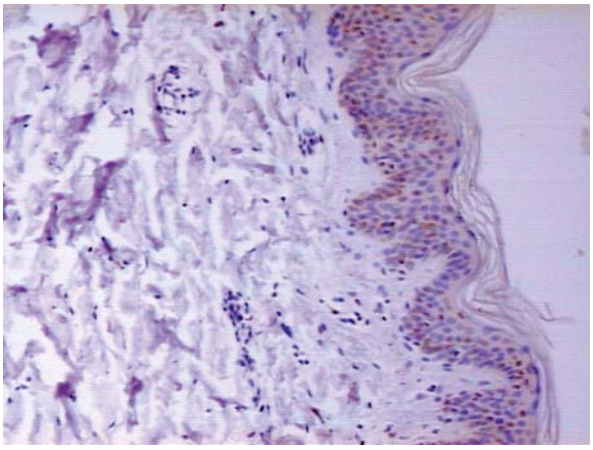

Fig. 7. TIMP 3 (100x). Skin lesions. Weak expression of TIMP 3 in perivascular infiltrates and in basal keratinocytes (mag. 100x)

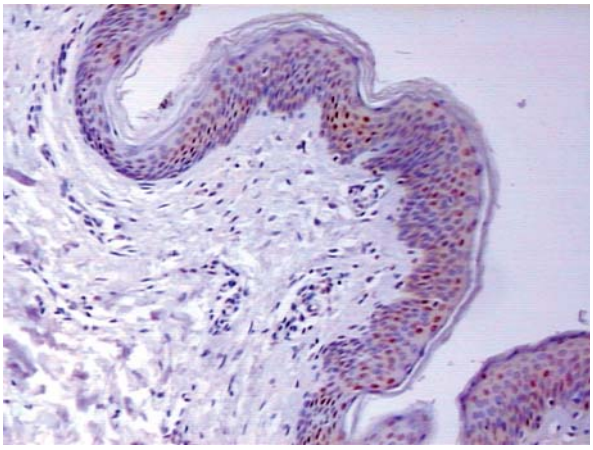

Fig. 8. Control (100x). Control. Moderate expression of MMP 1 the epidermis (mag. 100x)

[20, 32]. MMP 2, produced by fibroblasts and macrophages, cleaves gelatins, type VI collagen, fibronectins and proteoglycans [3].

Previous studies aimed at explanation of the role of metalloproteinases and their tissue inhibitors in pathogenesis and activity of systemic sclerosis were based on determination of their serum concentrations or expression in fibroblast cultures.

Literature data stress the important role of tissue inhibitors of metalloproteinases in development of fibrosis in the course of systemic sclerosis. Kikuchi et al. [29, 33] observed a correlation between serum TIMP concentration and disease activity in SSc patients. These authors also suggest that tissue inhibitor of metalloproteinase 1 has an important, autocrine role in fibrosis process. Matilla et al. [30] indicate a certain role of TIMP-3 in the process of degradation of extracellular matrix fibers in SSc. Yazawa et al. [3], on the other hand, observed an association between elevated concentration of tissue inhibitor of metalloproteinase 2 and pulmonary lesions in patients with systemic sclerosis.

Up to date, however, no studies were aimed at determination of degree of MMPs and TIMPs expression in lesional skin biopsies obtained from systemic sclerosis patients.

In our study pronounced expression of both metalloproteinases and their tissue inhibitors was demonstrated in skin biopsies from SSc patients in comparison to biopsies from healthy volunteers. Localization of examined enzymes was quite characteristic, concerning mostly dermal blood vessels and epidermis. Such expression of MMPs and TIMPs may be explained by their production by keratinocytes and inflammatory cells in the course of systemic sclerosis, while the presence of examined enzymes in the dispersed, parenchymal infiltrates in the dermis may confirm their role in degradation of ECM.

It would be interesting to explain why expression of selected metalloproteinases in SSc biopsies was observed in the same localization in the epidermis as Fas-APO1 in earlier studies [34]. It was demonstrated that both MMPs and their tissue inhibitors play a role not only in ECM degradation, but also other important processes, such as apoptosis [35]. In focal cerebral ischemia, for example, MMP 9 directly influenced the process of apoptosis in neurons, thus causing brain damage [36]. Authors of the other study demonstrated that intracellular gelatinases, MMP 2 and MMP 9, possess the ability to cut certain substrates, necessary for cell survival [37]. These observations may point to presence of distinct relation of metalloproteinases with suicidal cell death.

Intensity of gelatinase (MMP 2) and stromelysin (MMP 10) expression was strong, while MMP 1 and MMP 9 were moderately expressed. Expression of tissue inhibitors of MMPs was moderate or weak. It can be suspected that increased expression of most examined metalloproteinases in dermal blood vessels confirms their significant role in degradation of components of subendothelial basement membrane. This phenomenon may in turn facilitate migration of T cells into surrounding tissues, where they can indirectly influence increased production of ECM components by fibroblasts. Other studies reveal that expression of gelatinases (MMP 2 and MMP 9) facilitates migration of T cells through subendothelial basement membrane [38]. Activated T cells may directly, through cell-cell interaction, induce expression of metalloproteinase 9 in fibroblasts, neutrophils and monocytes [39-41].

In our study no association between intensity of expression of metalloproteinases 1, 2, 9 and 10 and duration of the Raynaud's phenomenon or duration of diseases was found. Results of other studies confirm the existence of such association between serum concentrations of metalloproteinases. Kuroda and Shinkai [42] revealed the presence of relationship between expression of genes for metalloproteinases and disease duration (in vitro studies – comparison of fibroblasts from systemic sclerosis patients and healthy persons).

No correlation between expression of MMPs/TIMPs and disease subset nor phase of disease was observed (data not shown).

Mechanism of disturbed balance between metalloproteinases and their tissue inhibitors causing excessive fibrosis in systemic sclerosis cannot be fully explained. It is suggested that excessive production of ECM may result from inhibition of MMPs' activity. Results of some studies demonstrate the role of antibodies directed against metalloproteinases 1 and 3, found in serum of systemic sclerosis patients [43-46].

Few studies evaluating the role and expression of metalloproteinases and their tissue inhibitors were so far conducted in bullous dermatoses [24, 47] and neoplastic diseases [48].

The hypothesis stating that influence of MMPs and TIMPs on ECM composition results not only from regulation of their production by fibroblasts, but also their direct role in the inflammatory process, may be confirmed by the fact of presence of significant MMP 1, MMP 2, MMP 10 and TIMP 2 expression in cells forming the inflammatory infiltrate – monocytes and lymphocytes. It seems also that lower expression of tissue inhibitors in comparison to metalloproteinases may indicate that imbalance between these enzymes influences increased tissue fibrosis in the course of systemic sclerosis. Similar disproportions in expression of MMPs and TIMPs were also found in studies on patients with Crohn disease [49], pemphigoid and Duhring disease [24], allowing the authors to form a hypothesis of their certain role in pathogenesis of these diseases.

The obtained results, although performed on a relatively small group of patients with systemic sclerosis, reveal the necessity of further explanation. They confirm the important role of imbalance between factors degrading the components

of extracellular matrix and their inhibitors, determined not only in sera, but also in tissue, in development of tissue fibrosis in systemic sclerosis.

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References

1. Valentini G, Della Rossa A, Bombardieri S, et al. (2001): European multicentre study to define disease activity criteria for systemic sclerosis. II. Identification of disease activity variables and development of preliminary activity index. Ann Rheum Dis 60: 592-598.
2. Varga J, Bashey RI (1995): Regulation of connective tissue synthesis in systemic sclerosis. Int Rev Immunol 12: 187-199.
3. Yazawa N, Kikuchi K, Ihn H, et al. (2000): Serum levels of tissue inhibitor of metalloproteinases 2 in patients with systemic sclerosis. J Am Acad Dermatol 42: 70-75.
4. Saarialho-Kere U, Kerkela E, Jahkola T, et al. (2002): Epilysin (MMP-28) expression is associated with cell proliferation during epithelial repair. J Invest Dermatol 119: 14-21.
5. Krampert M, Bloch W, Sasaki T, et al (2004): Activities of matrix metalloproteinase stromelysin-2 (MMP-10) in matrix degradation and keratinocyte organization in wounded skin. Mol Biol Cell 15: 5242-5254.
6. Fini ME, Cook JR, Mohan R, et al. Regulation of matrix metalloptoteinase gene expression. In: Matrix metalloproteinases. Ed. W Parks, R Mechan. Academic Press, San Diego. 1998, 300-356.
7. Bogaczewicz J, Chodorowska G, Krasowoska D (2003): Rola metaloproteaz macierzy i tkankowych inhibitorów metaloproteaz w twardzinie układowej. Przegl Dermatol 5: 373-381.
8. Greene J, Wang M, Liu YE, et al. (1996): Molecular cloning and characterization of human tissue inhibitor of metalloproteinase 4. J Biol Chem 271: 30375-30380.
9. Carmichael DF, Stricklin GP, Stuart JM (1989): Systemic administration of TIMP in the treatment of collagen-induced arthritis in mice. Agents Actions 27: 378-379.
10. Lee MM, Yoon BJ, Osiewicz K, et al. (2005): Tissue inhibitor of metalloproteinase 1 regulates resistance to infection. Infect Immun 73: 661-665.
11. Bogaczewicz J, Chodorowska G, Krasowska D (2004): Rola metaloproteinaz macierzy i tkankowych inhibitorów metaloproteinaz w progresji nowotworów skóry a nowe strategie farmakologicznej inhibicji metaloproteinaz macierzy. Przegl Dermatol 91: 153-160.
12. Wang JC (2005): Importance of plasma matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinase (TIMP) in development of fibrosis in agnogenic myeloid metaplasia. Leuk Lymphoma 46: 1261-1268.
13. Pesta M, Holubec L Jr, Topolcan O, et al. (2005): Quantitative estimation of matrix metalloproteinases 2 and 7 (MMP-2, MMP-7) and tissue inhibitors of matrix metalloproteinases 1 and 2 (TIMP-1, TIMP-2) in colorectal carcinoma tissue samples. Anticancer Res 25: 3387-3391.
14. Gu ZD, Li JY, Li M, et al. (2005): Matrix metalloproteinases expression correlates with survival in patients with esophageal squamous cell carcinoma. Am J Gastroenterol 100: 1835-1843.
15. Bjornland K, Flatmark K, Pettersen S, et al. (2005): Matrix metalloproteinases participate in osteosarcoma invasion. J Surg Res 127: 151-156.
16. Redondo P, Lloret P, Idoate M, et al. (2005): Expression and serum levels of MMP-2 and MMP-9 during human melanoma progression. Clin Exp Dermatol 30: 541-545.
17. Fatar M, Stroick M, Griebe M, et al. (2005): Matrix metalloproteinases in cerebrovascular diseases. Cerebrovasc Dis 20: 141-151.
18. Annala A, Koivukangas V, Salmela P, et al. (1995): Collagen synthesis markers and matrix metalloproteinases 2 (MMP-2) and 9 (MMP-9) in the suction blister fluid and serum of juvenile diabetic patients. Eur J Dermatol 5/3: 247-252.
19. Pawankar R (2005): Mast cells in allergic airway disease and chronic rhinosinusitis. Chem Immunol Allergy 87: 111-129.
20. Rannou F, Francois M, Corvol MT, Berenbaum F (2006): Cartilage breakdown in rheumatoid arthritis. Joint Bone Spine 73: 29-36.
21. Toubi E, Kessel A, Grushko G, et al. (2002): The association of serum matrix metalloproteinases and their tissue inhibitor levels with scleroderma disease severity. Clin Exp Rheumatol 20: 221-224.
22. Fujiwara M, Muragaki Y, Ooshima A (2005): Keloid-derived fibroblasts show increased secretion of factors involved in collagen turnover and depend on matrix metalloproteinase for migration. Br J Dermatol 153: 295-300.
23. Laudanski P, Szamatowicz J, Ramel P (2005): Matrix metalloproteinas-13 and membrane type-1 matrix metalloproteinase in peritoneal fluid of women with endometriosis. Gynecol Endocrinol 21: 106-110.
24. Zebrowska A, Narbutt J, Sysa-Jedrzejowska A, et al. (2005): The imbalance between metalloproteinases and their tissue inhibitors is involved in the pathogenesis of dermatitis herpetiformis. Mediators Inflam 2005: 373-379.
25. Zebrowska A, Waszczykowska E, Joss-Wichman E (2004): The role of adamalisins (ADAMs) in destruction of anchoring fibers involved in pathogenesis of selected subepidermal bullous diseases. Centr Europ J Immun 3-4: 85-89.
26. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee (1980): Arthritis Rheum 23: 581-590.
27. Ouchterlony O: Diffusion-in-gel methods for immunological analysis. In: Progress in Allergy. Ed. P Callos, BH Waxman. Karger, New York. 1962, 30.
28. Takeda K, Hatamochi A, Ueki H, et al. (1994): Decreased collagenase expression in cultured systemic sclerosis fibroblasts. J Invest Dermatol 103: 359-363.
29. Kikuchi K, Kadono T, Furue M, et al. (1997): Tissue inhibitor of metalloproteinase 1 (TIMP-1) may be an autocrine growth factor in scleroderma fibroblasts. J Invest Dermatol 108: 281-284.
30. Mattila L, Airola K, Ahonen M, et al. (1998): Activation of tissue inhibitor of metalloproteinases-3 (TIMP-3) mRNA expression in scleroderma skin fibroblasts. J Invest Dermatol 110: 416-421.
31. Kahari VM, Saarialho-Kere U (1997): Matrix metalloproteinases in skin. Exp Dermatol 6: 199-213.
32. Bullen EC, Longaker MT, Updike DL, et al. (1995): Tissue inhibitor of matalloproteinase-1 is decreased and activated gelatinases are increased in chronic wounds. J Invest Dermatol 104/2: 236-240.

33. Kikuchi K, Kubo M, Sato S, et al. (1995): Serum tissue inhibitor of metalloproteinases in patients with systemic sclerosis. J Am Acad Dermatol 33: 973-978.
34. Waszczykowska E, Sysa-Jędrzejowska A, Dziankowska--Bartkowiak B, et al. (2001): Apoptosis in the skin of patients with autoimmune connective tissue disorders. Centr Eur J Immunol 26: 53-58.
36. Brauer PR (2006): MMPs-role in cardiovascular development and disease. Front Biosci 11: 447-478.
37. Gu Z, Cui J, Brown S, et al. (2005): A highly specific inhibitor of matrix metalloproteinase-9 rescues laminin from proteolysis and neurons from apoptosis in transient focal cerebral ischemia. J Neurosci 25: 6401-6408.
38. Pereira AM, Strasberg-Rieber M, Rieber M (2005): Invasion--associated MMP-2 and MMP-9 are up-regulated intracellularly in concert with apoptosis linked to melanoma cell detachment. Clin Exp Metastasis 22: 285-295.
39. Leppert D, Waubant E, Galardy R, et al. (1995): T cell gelatinases mediate basement membrane transmigration in vitro. J Immunol 154: 4379-4389.
40. Burger D, Rezzonico R, Li JM, et al. (1998): Imbalance between interstitial collagenase and tissue inhibitor of metalloproteinases 1 in synoviocytes and fibroblasts upon direct contact with stimulated T lymphocytes: involvement of membrane-associated cytokines. Arthritis Rheum 41: 1748-1759.
41. Zhang JH, Ferrante A, Arrigo AP, et al. (1992): Neutrophil stimulation and priming by direct contact with activated human T lymphocytes. J Immunol 148: 177-181.
42. Lacraz S, Isler P, Vey E, et al. (1994): Direct contact between T lymphocytes and monocytes is a major pathway for induction of metalloproteinase expression. J Biol Chem 269: 22027-22033.
43. Kuroda K, Shinkai H (1997): Gene expression of types I and III collagen, decortin, matrix metalloproteinases and tissue inhibitors of metalloproteinases in skin fibroblasts from patients with systemic sclerosis. Arch Dermatol Res 289: 567-572.
44. Sato S, Hayakawa I, Hasegawa M, et al. (2003): Function blocking autoantibodies against matrix metalloproteinase-1 in patients with systemic sclerosis. J Invest Dermatol 120: 542-547.
45. Nishijima C, Hayakawa I, Matsushita T, et al. (2004): Autoantibody against matrix metalloproteinase-3 in patients with systemic sclerosis. Clin Exp Immunol 138: 357-363.
46. Bogaczewicz J, Krasowska D, Stryjecka-Zimmer M, Chodorowska G (2005): High serum total concentration of matrix metalloproteinase-9 (pro-MMP-9 and MMP-9) in patients with systemic sclerosis. Przegl Dermatol 3: 217-223.
47. Yu Q, Stamenkovic I (2000): Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev 14: 163-176.
48. Bodemer C, Tchen SI, Ghomrasseni S, et al. (2003): Skin expression of metalloproteinases and tissue inhibitor of metalloproteinases in sibling patients with recessive dystrophic epidermolysis and intrafamilial phenotypic variation. J Invest Dermatol 121: 273-279.
49. Kirkegaard T, Hansen A, Bruun E, et al. (2004): Expression and localisation of matrix matalloproteinases and their natural inhibitors in fistulae of patients with Crohn's disease. Gut 53: 701-709.