Gelatinase A Activity in Dupuytren's Disease

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Purpose: Dupuytren's contracture is a fibroproliferative disorder of the hand characterized by an abnormal myofibroblast and fibroblast proliferation and extracellular matrix deposition leading to retraction and deformation of the palm. Recent studies have shown that molecules of extracellular matrix may coordinate morphogenesis, cell differentiation, and most importantly, fibrogenesis in tissue. Gelatinase A (MMP-2) is a member of the matrix metalloproteinase family of proteolytic enzymes that contribute to remodeling the extracellular matrix by degrading its components. The aim of this study was to determine the level of MMP-2 activation in the palmar fascia of patients with Dupuytren's contracture with reference to the clinical stages of disease progression and recurrence of the contracture after surgery.

Methods: The level of relative MMP-2 activation, expressed by the active to latent MMP-2 ratio, was investigated with use of zymography and computerized densitometry in 16 normal and 71 pathologic tissues characterizing different clinical stages of the disease progression.

Results: We found that the level of MMP-2 activation was significantly elevated in the palmar fascias with Dupuytren's contracture compared with normal tissues. We did not find statistically significant differences between groups with different stages of the disease progression. We also did not find a relation between a high level of MMP-2 activation and the recurrence in the area of surgically treated Dupuytren's contracture.

Conclusions: The differences in MMP-2 activation between contractured and normal fascia suggest a participation of this enzyme in the promotion of Dupuytren's disease. We did not find a relationship, however, between the level of MMP-2 activation and the secondary contracture. (J Hand Surg 2006;31A:1635–1639. Copyright © 2006 by the American Society for Surgery of the Hand.)

Key words: Collagenase IV, gelatinase A, MMP-2, Dupuytren's contracture, matrix metalloproteinase.

Dupuytren's disease is a connective tissue disorder viewed as a progressive pathologic process involving multiple molecular events that lead ultimately to considerable changes in cell phenotype and function and to the deposition of excess matrix proteins in the extracellular space of the palmar aponeurosis, resulting in a flexion deformity of the fingers and loss of hand function.[1] The initial stage of the disease process is characterized by the appearance of small nodular thickenings composed of proliferative hyperplastic cells, in most of profibrogenic, myofibroblastic. The nodules over time evolve toward large, hypocellular bands of contracted, collagen-rich cords.[2] The growing nodules and the arrangement of newly formatted fibers entail tissue reorganization coupled with degradation of the surrounding extracellular matrix (ECM). Loss tissue integrity, as a result of matrix degradation occurs predominantly as a consequence of the action of a group of enzymes called the matrix metalloproteinases (MMPs), a multigene family of zinc- and calcium-dependent endopeptidases that are able to digest a wide range of ECM and nonmatrix compounds.[3]

Matrix metalloproteinase action has been implicated in both physiologic and pathologic tissue reshaping, such as organ development, wound repair, new vessel growth, inflammatory cell invasion, tumor infiltration, and metastases.[3–5] Activated MMPs degrading ECM molecules modulate the framework of matrix and cell behavior and survival by altering

cell ECM. Cell–cell interactions can also directly affect signaling through cleavage of signaling ligands and receptors.[6,7] At least 24 members of that family have been identified and classified into 2 structurally distinct groups, namely, secreted MMPs and membrane-type MMPs, containing a transmembrane domain.[8,9] Matrix metalloproteinase-2 (75-kDa gelatinase A), type IV collagenase, is an example of an enzyme secreted to the extracellular milieu that can digest denatured and native collagens including types I, IV, and V as well as fibronectin, elastin, or the protein core of some proteoglycans, among them decorin.[9–11]

Normally, MMP-2 has been associated with daily remodeling of the ECM.[12] It is produced mainly by fibroblasts and, like other members of the MMP family, is secreted in a latent form (pro-form), which requires cleavage of 80 amino acids of N-terminal segment to generate an active 62-kDa form.[13,14] Because the current evidence indicates that activated MMP-2 can have a dramatic effect on cell adhesion and proliferation and, as a stimulator of chemoattraction, may be critical for cell migration, it is accepted that this enzyme plays a prominent role in tumor growth.[15] On the other hand, there have been numerous reports[12,16–19] focused on the share of MMP-2 in various nontumorous disease states such as rheumatoid arthritis, atherosclerosis or fibrosis affected abnormally healed skin wounds, liver, lungs, heart, or kidney.

The aim of this study was to investigate the activation level of MMP-2, expressed by the percentage ratio of the active to latent forms, in palmar fascia with Dupuytren's contracture in relation to the clinical stages of disease progression. We determined the MMP-2 activation ratio from samples of pathologic aponeurosis and from normal fascia obtained from patients surgically treated for carpal tunnel syndrome. Using zymography, we found that MMP-2 is involved in the promotion of Dupuytren's contracture.

Materials and Methods

Fragments of pathologic palmar aponeurosis, taken during surgery from 71 patients (62 men, 9 women; age range, 33–72 y) who were treated surgically for Dupuytren's contracture between 1999 and 2005 at the Department of Traumatic Surgery and Hand Surgery, were the objects of the study. Iselin's classification[20] was used to identify the clinical stage of the disease progression: degree I, palmar nodules and small cords without signs of contracture in the interphalangeal joints; degree II, little contracture in the metacarpophalangeal and the proximal interphalangeal joints; degree III, contracture in the metacarpophalangeal, proximal interphalangeal, and distal interphalangeal joints; and degree IV, severe contracture in the metacarpophalangeal and proximal interphalangeal joints with hyperextension of the distal interphalangeal joints, together with advanced lesions in the osseous system. According to this classification, we examined palmar fascia tissue from 13 patients with disease degree I, 20 with degree II, 22 with degree III, and 16 with degree IV. For comparative purposes, we analyzed fragments of palmar fascia obtained from 16 patients (13 women, 3 men) who had surgery for carpal tunnel syndrome.

The levels of pro-form and active MMP-2 forms were determined from the tissue extracts. The tissue fragments were rinsed with 0.9% NaCl, dried on blotting paper, and homogenized in 1:10 of sample buffer (62.5 mmol/L tris–HCl with a pH of 6.8 with 10% glycerol, 2% sodium dodecyl sulfate and 0.05% bromophenol blue) in a glass Potter's homogenizer. After 30 minutes of incubation at 4°C homogenates were centrifuged for 15 minutes at 13,500 *g*. Gelanolytic activity in supernatants was determined with the use of the substrate gel sodium dodecyl sulfate–polyacrylamide gel electrophoresis zymography. Aliquots of the nonreduced samples (3–25 μL) were loaded in 7.5% polyacrylamide gels copolymerized with gelatine (2 mg/mL). After electrophoresis the enzymes were renatured by rinsing the sodium dodecyl sulfate twice in 50 mmol/L tromethamine–HCl with a pH of 7.5 with 2.5% Triton X-100 for 30 minutes at room temperature. Then gels were incubated for 20 hours at 37°C in 50 mmol/L tromethamine–HCl with a of pH 7.5 containing 150 mmol/L NaCl, 10 mmol/L $CaCl_2$, 1 μmol/L $ZnCl_2$, and 0.05% Brij-35. Gels were stained with 0.12% Coomassie Blue G-250 and cleared with mixture of 5% acetic acid and 10% ethanol to visualize the unstained proteolytic bands. The intensity of each sample band was (in inversion) determined densitometrically using software (ImageJ) and expressed as a ratio of the percentage of active to latent MMP-2 (activation ratio).

The results were analyzed statistically with the nonparametric Mann-Whitney *U* test and the Kruskal-Wallis test for comparing the 4 populations of patients with different disease stages. A p value of less than .05 was considered statistically significant.

We analyzed the rate of recurrence in the 36 patients who completed a questionnaire. The mean length of follow-up evaluation was 4 ± 1 y (range, 3–6 y). Of the 36 patients, 31 did not have recurrence

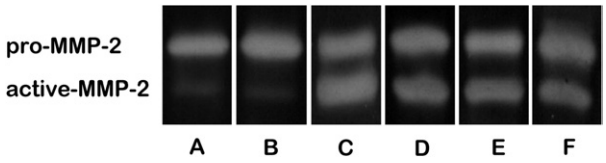

Figure 1. Gelatin zymogram showing MMP-2 pro- and active forms in tissue extracts of palmar fascia from 4 representative patients with Dupuytren's disease (lanes C–F) and from 2 healthy donors (lanes A, B). Lanes C through F show degrees I through IV of contracture, respectively.

during this time, and only 4 patients (11%) had recurrence in the treated area at a mean of 6 ± 3 months (range, 3–9 mo) after surgery. The chi-square test for a 2 × 2 contingency table was used to describe the relationship between the high (≥ 0.51) level of MMP-2 activation and the appearance of disease recurrence.

Results

Figure 1 shows a gelatin zymogram from normal and pathologic subjects randomly selected from the collection of all investigated samples. We found that the only gelatinolytic species, present in all tested tissues, were an active and a latent form of MMP-2.

In the group of 71 tested Dupuytren's specimens, the MMP-2 activation ratio had a median of 0.51 (range, 0.05–2.93) in contrast to the ratio in the 16 normal tissue specimens, which had a median of 0.075 (range, 0.03–0.21). The differences between both groups are significant ($p < .001$) (Fig. 2).

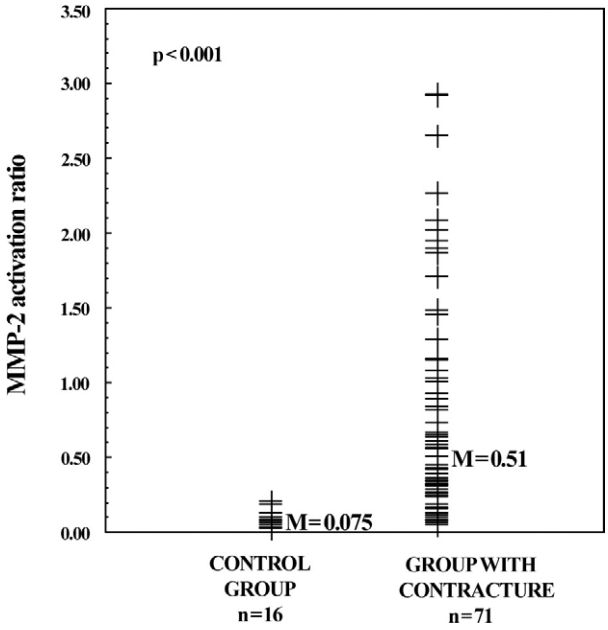

Figure 2. The MMP-2 activation ratios in normal fascia (control group) and in palmar aponeurosis with Dupuytren's contracture. M, median value.

Table 1. Medians and Ranges of MMP-2 Activation Ratio in the Group of Normal Tissues of Palmar Fascia (Control Group) and in Four Groups of Tissues of Palmar Aponeurosis with Dupuytren's Contracture in Dependence on Clinical Degree of Disease Progression– I°, II°, III°, and IV° According to Iselin

Group	MMP-2 Activation Ratio, Median (Range)	p
Control group (n = 16)	0.075 (0.030–0.210)	
Group I (n = 13)	0.640 (0.050–2.650)	<.001*
Group II (n = 20)	0.575 (0.070–2.930)	<.001*
Group III (n = 22)	0.355 (0.080–2.920)	<.001*
Group IV (n = 16)	0.345 (0.090–2.270)	<.001*

*Statistically significant difference.

Table 1 presents changes in the MMP-2 activation ratio depending on the clinical degree of Dupuytren's disease progression. The highest ratios were seen in the group of patients with degree I disease progression (median, 0.640) and the lowest ratios were observed in the group of patients with degree IV of clinical progression (median, 0.345). Significantly higher ratios in all clinical phases of the disease in relation to the control group were observed. The results of the Kruskal-Wallis test, however, indicated that groups of pathologic tissues with degrees I to IV did not differ significantly between each other ($p > .05$). In addition, because our chi-square statistic ($\chi 2 < .001$) did not exceed the critical value for the .05 probability level (3.841 for $df = 1$), we can accept the hypothesis that the recurrence of the contracture in surgically treated areas is independent of the high level of the MMP-2 activation ratio.

Discussion

The ECM is a dynamic complex mixture of various fibrillar proteins, primarily collagens, and nonfibrillar proteins interwoven into a network of glycosaminoglycan chains of proteoglycans, distributed in each organ in unique proportions adapted to the functional requirements of the particular tissues.[7] The macromolecules composing the ECM show a multifunctional nature. They are the scaffold for tissue formation and growth. Through direct binding cell receptors (integrins) they initiate signaling events to cell migration, proliferation, and differentiation. Extracellular matrix components can also selectively control the activity and presentation of a wide range

of growth factors.[7,21] Therefore, the ECM is important in the structure and in the function of all tissues, and even a slight alteration of its composition may have a dramatic effect on cellular behavior. The integrity of the ECM is controlled by a simple balanced equation of synthesis and degradation of ECM components.[21] This phenomenon is tightly coupled with functioning of the extracellular proteolytic system, which includes the activity of matrix metalloproteinases. A disturbance in secretion and activation of MMPs appears to play an important role in the development of numerous pathologic processes.[21] Palmar fibrosis, which is characterized by qualitative and quantitative alterations of ECM deposition, might result from insufficient matrix protein degradation. This report shows the relative MMP-2 activation level in aponeurosis with Dupuytren's contracture.

Although MMP-2 is the enzyme associated with continuous tissue remodeling, is necessary for the normal functioning of all tissues, and shows the most widespread expression among all MMPs, generally it is observed at low levels and mostly in the latent form.[9] In situations in which extensive matrix remodeling occurs—for instance, in repair processes or tumor growth and metastatic cascade including local invasion, angiogenesis, and extravasation—the level of MMP-2 activity rises.[17] Dupuytren's disease somewhat resembles benign tumorogenesis. Given that, it is not surprising that the palmar fascia tissues we investigated showed the increased gelatinolytic activity typical of cancerous processes. It is known, however, that an increase in MMP-2 activation coincides with ECM breakdown during tumor growth.[9]

In Dupuytren's aponeurosis a significantly increased MMP-2 activation ratio coexists with subsequent degradation of newly synthesized matrix components. Because the increase of MMP-2 activation was found in other fibrotic systems such as keloids and hypertrophic scars, it may be suggested that fibrosis does not arise from the loss of MMP-2 activation.[12] It is plausible, however, that during fibrosis a low efficiency of MMP-2 activity appears as an effect of a reduced ratio of MMP-2s to their inhibitors (TIMPs). The TIMPs are often upregulated when increased MMP activity occurs[16] and may prevent proteolytic cleavage of the proenzyme and function of the active enzyme.[22] Ulrich et al[23] have shown that tissues of patients with palmar fibrosis stained intensively positive for TIMP-1 and TIMP-2 when immunohistochemical methods were used. It is also plausible that MMP-2 is a potent promotor of fibrosis. The MMP-2 activation may have an important effect on the regulation of profibrotic transforming growth factor–β1 (TGF-β1), which was found at high levels in all stages of Dupuytren's disease.[24,25] Transforming growth factor–β1 is secreted and maintained in a latent complex with a small proteoglycan, decorin. This complex functions as a reservoir of TGF-β1 in the extracellular milieu.[11] Because decorin is susceptible to degradation by MMP-2 it might be suggested that MMP-2 releases TGF-β1 from the decorin–TGF-β1 complex and in that way plays a key role in the control of TGF-β1 activation and fibrosis promotion.

The aim of this study was to determine whether the level of MMP-2 activation correlates with the clinical stages of Dupuytren's disease progression. From a clinical point of view, there are 4 degrees in the course of Dupuytren's disease according to Iselin.[20] Each of them characterizes a different stage of the palm contracture and tissue architecture.[26] It has been reported[24] that the biosynthesis of both noncollagen and collagen proteins elevates in the initial phases of Dupuytren's disease and decreases during the final stage of fibrosis. This phenomenon correlates with the presence of myofibroblasts.[2,20] We showed that regardless of the clinical stage of disease progression, the activation ratios of MMP-2 remain significantly elevated even in the terminal phase of fibrosis when the cellular structure of the fascia returns to the state observed in the normal palmar aponeurosis. In this context, the activity of MMP-2 seems to be dependent on factors of nonmyofibroblastic origin. Robbins et al[27] reported that platelet-derived growth factor positively regulates MMP-2 expression and activation during normal development. Platelet-derived growth factor, known as a mitogen and potent chemoattractic agent for fibroblasts, is locally secreted by platelets and smooth muscle cells.[27] Because it was shown that platelet-derived growth factor is expressed at a higher level in Dupuytren's disease, we may conclude that MMP-2 activation might depend on this growth factor activity.[28]

The risk of recurrence is common and remains an obvious problem for up to 78% of surgically treated patients.[29] Each recurrence and each repeat surgery gives an increased probability of complications. Despite many investigations no trustworthy risk factor for the recurrence of Dupuytren's contracture has been identified.[29] We also did not find that a high level of MMP-2 activation might play a role in increased risk of disease recurrence.

This study shows unequivocally that activated MMP-2 is involved in the development of Dupuytren's contracture, but it does not have the prognostic value for predicting recurrence after surgery. The real role of this enzyme and its relations with other MMPs and growth factors in the pathogenesis of palmar fibrosis are subjects for future investigations.

Received for publication April 21, 2006; accepted in revised form August 15, 2006.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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0363-5023/06/31A10-0010$32.00/0
doi:10.1016/j.jhsa.2006.08.007

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