

Matrix Metalloproteinase Inhibition Reduces Contraction by Dupuytren Fibroblasts

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Purpose Dupuytren's disease is a common fibroproliferative condition of the hand characterized by fibrotic lesions (nodules and cords), leading to disability through progressive digital contracture. Although the etiology of the disease is poorly understood, recent evidence suggests that abnormal matrix metalloproteinase (MMP) activity may play a role in cell-mediated collagen contraction and tissue scarring. The aim of this study was to investigate the efficacy of ilomastat, a broad-spectrum MMP inhibitor, in an *in vitro* model of Dupuytren fibroblast-mediated contraction.

Methods Nodule-derived and cord-derived fibroblasts were isolated from Dupuytren patients; carpal ligament-derived fibroblasts acted as control. Stress-release fibroblast-populated collagen lattices (FPCLs) were used as a model of contraction. FPCLs were allowed to develop mechanical stress (48 hours) during treatment with ilomastat (0–100 $\mu\text{mol/L}$), released, and allowed to contract over a 48-hour period. Contraction was estimated by measuring lattice area compared with untreated cells or treatment with a control peptide. MMP-1, MMP-2, and MT1-MMP levels were assessed by zymography, Western blotting, and enzyme-linked immunosorbent assay.

Results Nodule-derived fibroblasts contracted lattices ($69\% \pm 2$) to a greater extent than did cord-derived ($55\% \pm 3$) or carpal ligament-derived ($55\% \pm 1$) fibroblasts. Exposure to ilomastat led to significant inhibition of lattice contraction by all fibroblasts, although a reduction in lattice contraction by nodule-derived fibroblasts was most prominent ($84\% \pm 8$). In addition, treatment with ilomastat led to a concomitant suppression of MMP-1 and MMP-2 activity, whereas MT1-MMP activity was found to be upregulated.

Conclusions Our results demonstrate that inhibition of MMP activity results in a reduction in extracellular matrix contraction by Dupuytren fibroblasts and suggest that MMP activity may be a critical target in preventing recurrent contracture caused by this disease. (*J Hand Surg* 2008;33A:1608–1616. Copyright © 2008 by the American Society for Surgery of the Hand. All rights reserved.)

Key words Dupuytren, ilomastat, MMP, recurrent contracture.

DUPUYTREN'S DISEASE IS a common fibroproliferative condition of the palmar fascia of the hand that causes disability through progressive digital contracture. Clinically, 2 forms of disease are

readily identifiable, namely nodules of hyperproliferative cells and collagen-dense cords. Although the disease pathogenesis is poorly understood, development of contracture is thought to occur through simultaneous

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Received for publication February 5, 2008; accepted in revised form June 12, 2008.

The authors wish to acknowledge the Royal College of Surgeons of England and the RAFT Institute.

Support was received from the Royal College of Surgeons of England.

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0363-5023/08/33A09-0022\$34.00/0
doi:10.1016/j.jhssa.2008.06.013

fibroblast contraction and remodeling of the surrounding matrix.¹ The result of these activities is a step-wise permanent shortening of the diseased tissue. Intervention is almost exclusively surgical but despite recent advances in operative technique, recurrence remains an unsolved problem.² Furthermore, nonoperative treatments such as off-label use of gamma-interferon have yet to gain widespread acceptance, although collagenase injection therapy is currently being investigated in a clinical trial.³

The matrix metalloproteinases (MMPs) are a group of zinc-dependent endopeptidases that cleave extracellular matrix (ECM) components and participate in many biological processes. Each MMP has distinct but often overlapping substrate-specificity, and together they can cleave all ECM proteins.⁴ Through matrix resorption, MMPs facilitate tissue remodeling, cell migration, and release of biologically active molecules that regulate fundamental cellular processes such as proliferation, growth, signaling, and differentiation.⁵ MMPs are secreted as inactive zymogens that are subsequently activated by cleavage of a prodomain. MMPs are usually present at low levels, and their expression is tightly regulated by an intricate, multidimensional system of activators and inhibitors including the tissue inhibitors of metalloproteinases, an important family of endogenous inhibitors.⁶ The net balance of MMP activity in the extracellular space is crucial for guiding normal biological processes such as embryogenesis and wound repair.^{4,7} Loss of control of MMP activity is implicated in numerous pathologic conditions including arthritis, chronic obstructive pulmonary disease, and atherosclerosis, as well as facilitating metastatic spread in cancer.⁸⁻¹¹ Indeed, MMP activity is an important therapeutic target in many diseases.^{12,13} There is also an accumulating body of evidence to suggest that abnormal MMP activity is a feature of Dupuytren's disease.^{14,15}

Fibroblast-populated collagen lattices (FPCLs) are a three-dimensional *in vitro* model system that simulate the interaction between cells and their surrounding matrix.¹⁶ Fibroblast-populated collagen lattices are therefore a valuable model for studying cell-mediated tissue organization. MMP activity has been shown to play a critical role in FPCL contraction *in vitro*.¹⁷⁻²⁰ Furthermore, MMP inhibition has been shown to reduce postoperative scarring in an experimental model of glaucoma filtration surgery.²¹ The current study was performed as a preliminary assessment of the therapeutic potential of ilomastat in preventing contracture. Ilomastat is a hydroxamate class of MMP inhibitor that binds reversibly to the zinc active site of several differ-

ent MMPs conferring broad-spectrum inhibition. Our specific aim was to investigate the effect of ilomastat on matrix contraction mediated by Dupuytren fibroblasts (nodule and cord) *in vitro* using an FPCL model.

MATERIALS AND METHODS

Cell culture

Five sets of paired Dupuytren cord and nodule fibroblasts were isolated by explant culture from excised specimens at elective primary surgical fasciectomy after local ethical committee approval. The Dupuytren tissue samples were harvested from 5 men of median age 64 years (range, 45-77 years). Nodule and cord tissue were distinguished clinically and by histologic evaluation.²² Carpal ligament was selected as control palmar fascia and excised at routine carpal tunnel decompression from patients (2 men, 3 women, median age 58 years) with no clinical evidence of Dupuytren's disease. Cells were maintained in normal growth medium (NGM) at 37°C with 5% CO₂. Normal growth medium consisted of Dulbecco's modified Eagle's medium (DMEM; Gibco, Paisley, UK) supplemented with Glutamax 2 mmol/L, 10% (vol/vol) fetal calf serum (FCS), penicillin (100 U/mL), and streptomycin (100 µg/mL; all Gibco). Gelatinase-free media (GFM) was prepared by substituting gelatinase-free FCS for normal FCS. The FCS was made gelatinase-free by incubation with gelatin Sepharose 4B (Amersham Biosciences, Amersham, UK) at 4°C on a vertical rotary shaker for 2 hours. The subsequent mixture was centrifuged at 1,000 rpm for 5 minutes and the supernatant (gelatinase-free FCS) aspirated and used to make GFM. Only cells between passages 3 and 5 were used for the experiments.

Ilomastat (GM6001; Calbiochem, Nottingham, UK) and the control peptide (GM6001 negative control; Calbiochem) were prepared in dimethyl sulfoxide (DMSO; Sigma Chemical Co., Dorset, UK) at 100 mmol/L as both reagents are poorly soluble in aqueous solutions. The control peptide is identical to ilomastat in structure but lacks the active site and therefore enzyme activity. Serial dilutions of ilomastat, the control peptide, and vehicle control (DMSO) were used in subsequent experiments as described.

Fibroblast proliferation

Fibroblasts were seeded into 96-well tissue culture plates at a density of 4×10^3 cells per well in NGM and incubated at 37°C to allow cells to adhere. After 24 hours, the culture media was removed and replaced by fresh NGM supplemented with serial dilutions of ilomastat (0, 1 nmol/L, 10 nmol/L, 100 nmol/L, 1 µmol/L,

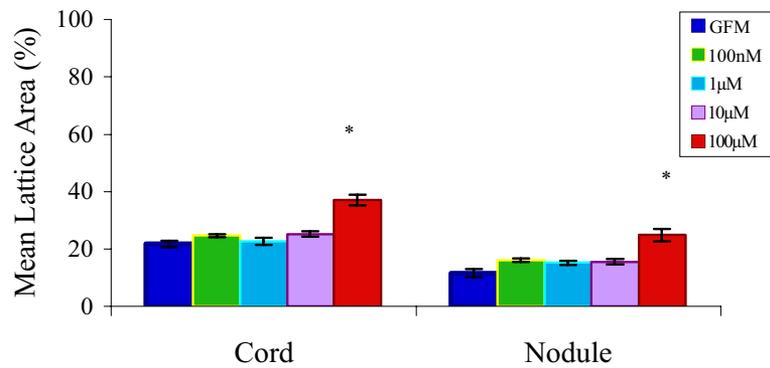


FIGURE 1: Effect of serial dilutions of ilomastat (100 nmol/L to 100 μ mol/L) on lattice contraction at 48 hours by cord-derived and nodule-derived fibroblasts ($n = 4$ replicates). * $p < .001$ represents a significant difference between ilomastat and NGM treatment. Mean \pm SEM.

10 μ mol/L, 100 μ mol/L, 1 mmol/L), control peptide, or vehicle control (DMSO 0, 0.000001%, 0.00001%, 0.0001%, 0.001%, 0.01%, 0.1%, 1%). Cell number was estimated at 0, 24, and 48 hours after exposure using the WST-1 assay according to the manufacturer's instructions (Roche Molecular Diagnostics and Biochemicals, Lewes, UK). Changes in cell number in response to ilomastat, control peptide, and vehicle control were compared with responses by cells cultured in NGM alone.

Fibroblast-populated collagen lattice

Matrix contraction was assessed using a stress-release FPCL model. Cord-seeded and nodule-seeded FPCLs were initially exposed to serial dilutions of ilomastat (0, 100 nmol/L, 1 μ mol/L, 10 μ mol/L, 100 μ mol/L). This preliminary work established that 100 μ mol/L was the lowest effective dose at mediating suppression of lattice contraction (Fig. 1). In this study, three-dimensional type I collagen (Sigma Chemical Co.) lattices were prepared as follows. The lattice mixture was prepared by mixing 350 μ L 10x DMEM (Gibco) and 600 μ L 5 mg/mL type I collagen (Sigma) with 150 μ L cell suspension (final concentration 5×10^5 fibroblasts/mL of lattice). The solution was neutralized by dropwise addition of 0.1 mol/L NaOH and 150 μ L lattice mixture dispensed per well of a 48-well plate. Lattices were incubated for 1 hour at 37°C to allow polymerization. Fibroblast-populated collagen lattices were allowed to develop mechanical stress for 48 hours in response to ilomastat (100 μ mol/L), control peptide, or vehicle control (0.1% DMSO). After 48 hours, the media (GFM \pm ilomastat, peptide, or vehicle alone) was replaced, and lattices were released and allowed to contract over a further 48-hour period. Lattices were digitally recorded and contraction estimated by measuring change in lat-

tice area over time (ImageTool 3.0; Evans Technology, Roswell, GA). Treatment with ilomastat was compared with control peptide or untreated lattices. Cell-conditioned media samples were collected both under conditions of mechanical stress (before release) and lattice contraction (48 hours after release) and stored at -20°C for subsequent analysis. Cells were retrieved from lattices by collagenase digestion (0.5% collagenase D, Roche; 0.5% bovine serum albumin, Sigma; phosphate-buffered saline) at 37°C for 20 minutes. Cells were concentrated by centrifugation and lysed in 100 μ L lysis buffer (50 mmol/L tris-buffered saline pH 7.6, 1.5 mmol/L NaCl, 0.5 mmol/L CaCl_2 , 1 μ mol/L ZnCl_2 , 0.1% Brij-35, 0.25% Triton X-100) to isolate membrane-bound and intracellular proteins. Lysates were stored at -20°C .

Estimation of protein content

The total protein concentration of samples (conditioned media and cell lysates) derived from FPCL experiments was estimated using a commercial reagent based on the Bradford Protein Assay (BioRad, Hemel Hempstead, UK). Levels of MMP protein and enzyme activity were subsequently normalized against total protein concentration.

Estimation of MMP secretion

Western blot analysis was performed to investigate the presence of MMP-1 and MMP-2 levels in conditioned media derived from FPCL experiment as both MMPs are expressed by Dupuytren tissue and have been shown to be important in fibroblast-mediated lattice contraction.^{18,20,23} Levels of MT1-MMP, a membrane-bound activator of proMMP-2 with collagenase activity, were determined in cell lysate samples. A volume yielding 20 μ g (MMP-1, MMP-2) or 5 μ g protein

(MT1-MMP levels) was diluted 1:1 with sample buffer. After sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electroblotting, nitrocellulose membranes were blocked with a 5% bovine serum albumin solution in tris-buffered saline prior to incubation with specific anti-MMP antibodies (1:1000 mouse anti-MMP-1; 1:1000 mouse anti-MMP-2, Calbiochem, UK; 1:1000 rabbit anti-MT1-MMP, Chemicon, Consett, UK) overnight at room temperature and subsequently for 1 hour with a secondary horse radish peroxidase–conjugated antibody (1:1000 goat anti-mouse, Calbiochem; 1:1000 goat anti-rabbit, Sigma). Detection was performed using an alkaline phosphatase reagent.

Quantification of MMP activity

Gelatin zymography: Conditioned media samples were analyzed by gelatin zymography. Sample kaleidoscope markers (14.3–220 kDa) were dispensed into the inside lane of each zymogram to detect characteristic MMP molecular weight shifts after activation. A volume yielding 20 μg total protein was loaded into successive lanes and the zymogram run at 25 mA for 150 minutes. Zymogram gels were developed overnight at 37°C using a commercial system of buffers (BioRad) in accordance with the manufacturer's instructions, then stained with 0.5% Coomassie brilliant blue R-250 (Sigma). After careful destaining of the zymogram (10% acetic acid, 40% methanol, 50% distilled H₂O), gelatinolytic bands corresponding with enzyme activity were revealed.

Enzyme-linked immunosorbent assay (ELISA): Active MMP-1 levels were determined in conditioned media collected from contracting FPCLs using a commercially available ELISA kit (Amersham Biosciences). An ELISA assay (Amersham) was also used to determine active MT1-MMP levels in diluted (10-fold) cell lysate samples. Each ELISA was carried out using instructions provided by the manufacturer to determine native enzyme activity levels. The MMP detection range of each assay was as follows; 0.1 to 1.56 ng/mL (MMP-1) and 1 to 32 ng/mL (MT1-MMP), respectively.

Statistical analysis

Statistical analysis was performed using a statistical software package (SigmaStat 2.0; SPSS, Chicago, IL). A two-tailed Student's *t*-test was used to compare treatments between 2 groups, and a 1-way analysis of variance (ANOVA) was used to compare results derived from multiple groups. Probabilities with $p < .05$ were considered statistically significant.

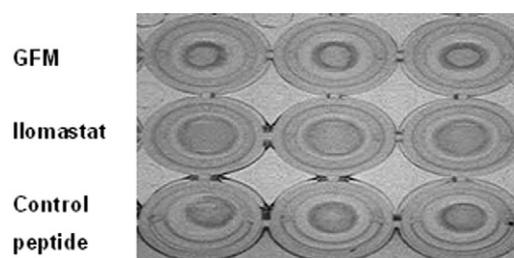


FIGURE 2: Photograph illustrating actively contracting collagen lattices in response to GFM or control peptide (100 $\mu\text{mol/L}$; top and bottom rows, respectively) compared with inhibition of contraction by cells in response to ilomastat (100 $\mu\text{mol/L}$; central row).

RESULTS

Effect of ilomastat on fibroblast proliferation

The proliferation of fibroblasts exposed to concentrations of ilomastat (1 nmol/L to 1 mmol/L) was compared with proliferation of cells cultured in NGM or vehicle control. After a 48-hour exposure to 100 $\mu\text{mol/L}$ ilomastat, fibroblast proliferation, expressed as a change in optical density, did not appear to be significantly different than that of fibroblasts cultured in NGM (OD 1.11 ± 0.05 and 1.08 ± 0.04 , respectively) or in NGM with vehicle control (0.1% DMSO, OD 1.16 ± 0.04). Fibroblast proliferation was significantly decreased when cells were exposed to ilomastat at concentrations greater than 100 $\mu\text{mol/L}$ ($p < .05$), and when exposed to DMSO control (1%, $p < .05$), compared with NGM. These results suggested that the inhibition of fibroblast proliferation was mediated by the concentration of the DMSO in preparations of the vehicle control and ilomastat rather than by any ilomastat toxicity. Subsequent experiments used 100 $\mu\text{mol/L}$ ilomastat as the optimum concentration at which fibroblasts remained viable and functional; this concentration of ilomastat contained less than 0.1% DMSO, a nontoxic level.

Effect of ilomastat on fibroblast-mediated collagen contraction

Fibroblasts derived from cord, nodule, and carpal ligament all demonstrated an ability to contract FPCLs in a stress-release model of contraction. Figure 2 illustrates actively contracting collagen lattices in response to GFM or control peptide (100 $\mu\text{mol/L}$; top and bottom rows respectively) compared with inhibition of contraction by cells in response to ilomastat (central row). Lattice contraction by all cell types (Fig. 3) appeared maximal at 48 hours after release, and whereas the magnitude of contraction

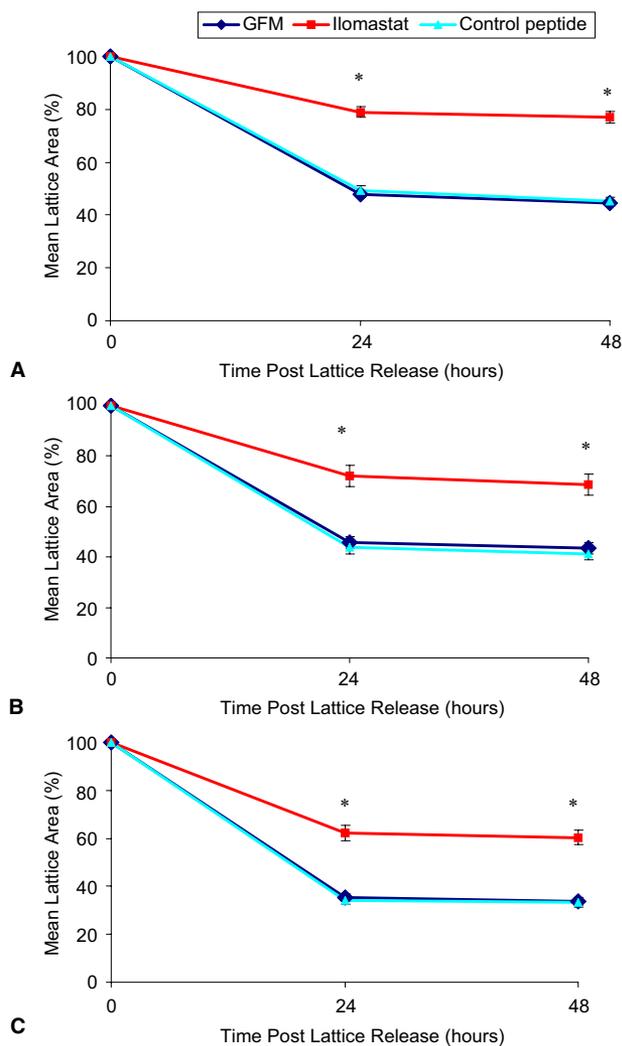


FIGURE 3: Fibroblast-mediated collagen lattice contraction. Panels **A** to **C** illustrate lattice contraction by cells derived from carpal ligament (**A**; $n = 5$), Dupuytren cord (**B**; $n = 5$), and nodule (**C**; $n = 5$). * $p < .001$ represents a significant difference between ilomastat exposure and GFM or control peptide. Mean \pm SEM.

varied depending on the origin of the cell type, GFM-mediated contraction did not appear to be significantly different than contraction exhibited in response to the control peptide. Exposure to ilomastat ($100 \mu\text{mol/L}$) resulted in a significant suppression of FPCL contraction compared with GFM ($p < .001$) in all 3 fibroblast types assessed.

Nodule-derived fibroblasts contracted lattices by $66\% \pm 2$, a significantly greater extent than cord-derived or carpal ligament-derived fibroblasts ($57\% \pm 2$ and $55\% \pm 1$, respectively; $p < .05$) under basal conditions (Fig. 4A). Treatment with ilomastat inhibited lattice contraction by carpal ligament-derived ($73\% \pm 6$), cord-derived ($63\% \pm 7$), and nodule-

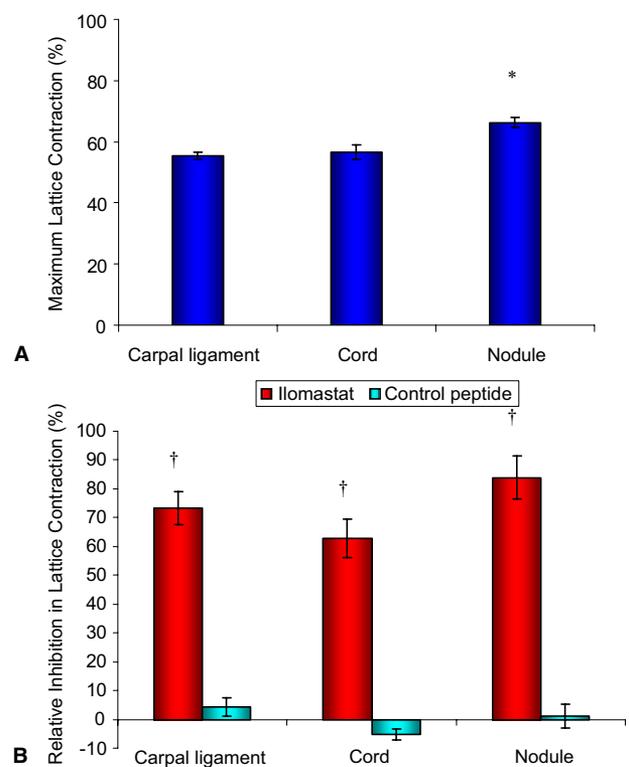


FIGURE 4: Differential contractile activity of Dupuytren-derived and carpal ligament-derived fibroblasts. **A** A comparison of contractile activity of all 5 cell strains derived from carpal ligament and Dupuytren tissue (cord and nodule) in response to GFM. * $p < .05$ represents a significant difference between nodule and both cord and carpal ligament. **B** Inhibition by ilomastat and control peptide relative to GFM treatment is demonstrated, ascribed a value of 0. † $p < .05$ represents a significant difference between ilomastat and GFM treatment. Mean \pm SEM.

derived fibroblasts ($84\% \pm 8$) compared with treatment with control peptide ($p < .001$; Fig. 4B). Nodule-derived fibroblasts appeared to be more sensitive to inhibition by ilomastat than were cord-derived or carpal ligament-derived fibroblasts although the differences proved not to be significant.

Effect of ilomastat on MMP secretion and expression

The effect of ilomastat treatment on secretion of a range of MMPs and expression of MT1-MMP was assessed *in vitro* (Fig. 5). After release of prestressed lattices, carpal ligament-derived, cord-derived, and nodule-derived fibroblasts appeared to secrete similar levels of MMP-1 and MMP-2 as determined by Western blot analysis. Both pro (55 kDa) and active (43 kDa) forms of MMP-1 were detected. By contrast, only the pro form of MMP-2 was detected by Western blotting. Treatment with ilomastat or the

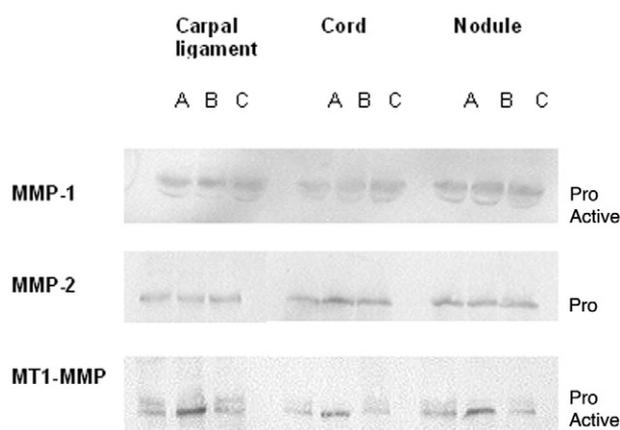


FIGURE 5: Analysis of membrane-bound (MT1-MMP) and secreted MMPs (MMP-1, MMP-2). Western blot analysis of MMP production by carpal ligament–derived, cord–derived, and nodule–derived fibroblasts seeded within contracting (after release) lattices exposed to either **A** GFM, **B** ilomastat, or **C** control peptide.

control peptide (100 $\mu\text{mol/L}$) did not appear to significantly affect MMP secretion, as assessed by scanning densitometry (data not shown). Carpal ligament–derived, cord–derived, and nodule–derived fibroblasts expressed both pro (65 kDa) and active (63 kDa) forms of MT1-MMP, a membrane-bound MMP, after release of prestressed lattices. Exposure to ilomastat (100 $\mu\text{mol/L}$) resulted in an upregulation of active MT1-MMP by carpal ligament–derived, cord–derived, and nodule–derived fibroblasts. Treatment with control peptide did not appear to significantly affect MT1-MMP expression.

Effect of ilomastat on MMP activity

Gelatin zymography allowed estimation of the level of enzyme activity of a range of secreted and membrane-bound MMPs (Fig. 6). In stressed lattices (before release), carpal ligament–derived, cord–derived, and nodule–derived fibroblasts released similar levels of a gelatinolytic species that corresponded with proMMP-2 (72 kDa; Fig. 6). Treatment with ilomastat or the control peptide did not significantly affect proMMP-2 activity. After release of stressed lattices, all Dupuytren–derived and non–Dupuytren–derived fibroblasts secreted both proMMP-2 and an additional gelatinolytic species corresponding with active MMP-2 (66 kDa). Treatment with ilomastat appeared to markedly inhibit the enzyme activity associated with activated MMP-2 in all cell strains assessed. MMP-9, another important gelatinase, was not detected by zymography.

To quantify ilomastat-mediated alterations in MMP enzyme activity, conditioned media and cell lysates collected from contracting FPCLs were subjected to an ELISA (Fig. 7). Carpal ligament–derived and cord–derived fibroblasts secreted similar levels of active MMP-1 (0.4 ng/mg \pm 0.06 and 0.39 ng/mg \pm 0.02, respectively) in contracting lattices (Fig. 7A). By contrast, nodule–derived fibroblasts appeared to secrete significantly higher levels (1.25 ng/mg \pm 0.18; $p < .05$) of active MMP-1. This activity was significantly inhibited by exposure to ilomastat, leading to a 47% reduction in active MMP-1 compared with MMP-1 secreted in response to GFM alone ($p < .05$). Ilomastat also suppressed MMP-1 activity from cord–derived fibroblasts. MMP-1 activity was not significantly affected by exposure to the control peptide.

Even though all cells assessed demonstrated measurable amounts of MT1-MMP in response to GFM basal media, cord–derived and nodule–derived cells appeared to express higher basal levels (Fig. 7B). Ilomastat treatment appeared to increase MT1-MMP activity in nodule and carpal ligament cells, corresponding with suppression of lattice contraction. Nodule–derived fibroblasts exhibited the greatest increase in MT1-MMP expression, with levels rising from 212 ng/mg \pm 9 to 630 ng/mg \pm 40 ($p < .001$). Treatment with control peptide did not significantly alter activity of MT1-MMP.

DISCUSSION

Dupuytren's disease is a condition characterized by contractile scarring of the palmar fascia. Fibroblast proliferation, contraction, and matrix deposition are key processes identified by *in vitro* studies that may be important in the pathogenesis of contracture development.^{24–26} The FPCL is a commonly used *in vitro* model of tissue organization that was employed in this study to assess the contractile behavior of Dupuytren fibroblasts. Specific cellular activities including fibroblast migration, differentiation to myofibroblasts, and matrix remodeling are thought to be fundamental to lattice contraction in the FPCL model.¹⁶ In our study, the role of MMP activity in collagen lattice contraction by Dupuytren fibroblasts was investigated using ilomastat, a broad-spectrum MMP inhibitor. Nodule–derived and cord–derived fibroblasts were cultured and investigated separately as previous studies have demonstrated distinct histologic profiles for the two clinical phenotypes. Nodule tissue is highly cellular and rich in myofibroblasts compared with normal palmar fascia or cord.^{22,27,28}

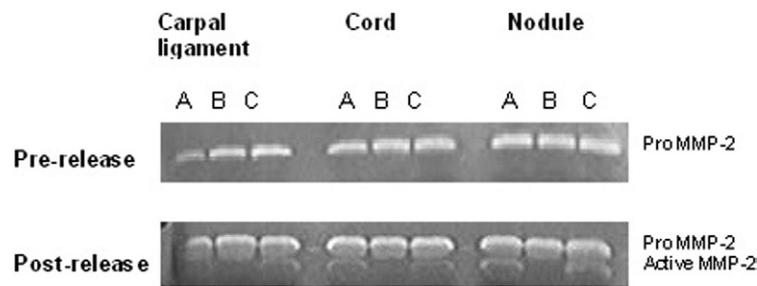


FIGURE 6: Analysis of gelatinolytic activity produced in conditioned media collected from stressed (before release) and contracting (after release) collagen lattices. Carpal ligament–derived, cord-derived, and nodule-derived fibroblasts were exposed to either **A** GFM, **B** ilomastat, or **C** control peptide.

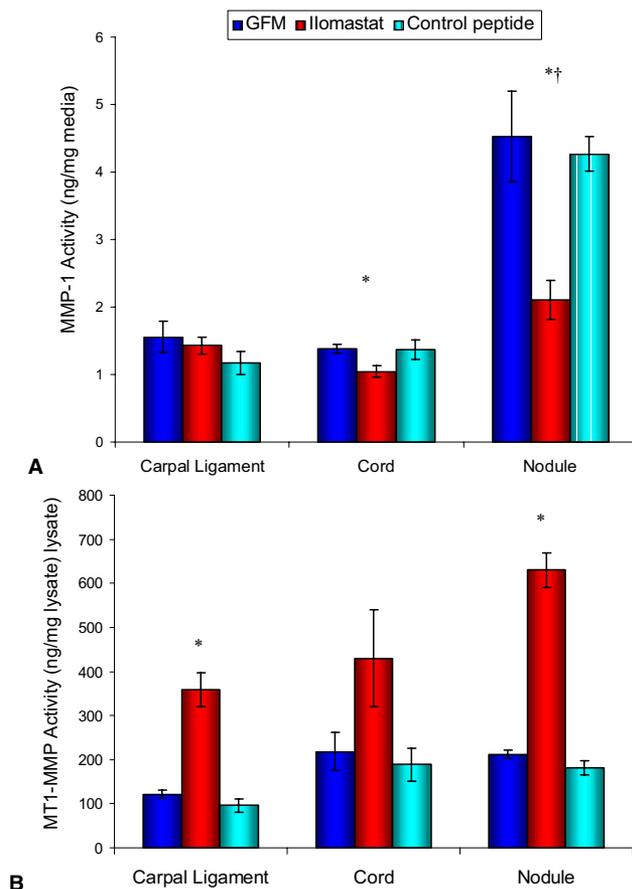


FIGURE 7: Quantification of MMP-1 and MT1-MMP activity in response to ilomastat. **A** MMP-1 activity (ng/mg) in conditioned media during FPCL contraction in response to GFM, ilomastat, or control peptide. **B** MT1-MMP activity (ng/mg cell lysate) during FPCL contraction. * $p < .05$ represents a significant difference between GFM and ilomastat treatment. † $p < .05$ represents a significant difference between nodule and cord or carpal ligament. Mean \pm SEM.

Nodule-derived fibroblasts demonstrated a greater contractile ability compared with cord-derived or carpal ligament–derived fibroblasts in the FPCL model, a

finding consistent with a previous study.²⁴ Treatment with ilomastat significantly reduced collagen lattice contraction by Dupuytren (nodule and cord) as well as carpal ligament–derived fibroblasts. This was unrelated to cytotoxicity as cells were able to proliferate normally and remain viable at the concentration of ilomastat investigated, 100 μ mol/L. In addition, inhibition of lattice contraction by ilomastat was more pronounced in nodule-derived than in cord-derived fibroblasts, although this difference was not statistically significant. Clinically, nodules are thought to precede cords and may therefore be the initial fuse to contracture development.²⁹ Our results demonstrate that nodule-derived fibroblasts mediate greater lattice contraction than do cord-derived fibroblasts and were similarly sensitive to inhibition by ilomastat. These findings imply that intervention with an MMP inhibitor might be best directed at an early stage of disease, when nodules predominate. By specifically targeting nodules, it may be possible to prevent the progression to contracture and avoid the need for surgery.

Analysis of media samples collected during lattice contraction demonstrated involvement of several MMPs in this process. Fibroblasts seeded in contracting lattices (after release) produced MMP-1, MMP-2, and MT1-MMP. The primary substrate of MMP-1 is collagen type I, and similar to the findings of previous investigators, MMP-1 activity was detected in the media collected from contracting FPCLs.^{20,30} In our study, MMP-1 activity was most prominent in nodule-derived fibroblasts, which also contracted lattices to the greatest extent. Elevated levels of MMP-1 mRNA in Dupuytren-derived nodule have also been observed in palmar aponeurosis.³¹ In our study, treatment with ilomastat inhibited enzymic activity of MMP-1 in Dupuytren-derived fibroblasts (both nodule and cord) whereas levels of secreted MMP-1 protein were unaffected. These results imply utilization of MMP-1 during matrix

contraction, a finding supported by a previous study using ocular fibroblasts.¹⁸ Surprisingly, ilomastat did not affect MMP-1 activity in control carpal ligament fibroblasts as a group, although some individual carpal ligament strains demonstrated a notable inhibition in response to ilomastat. A definitive pattern of inhibition may have been seen with a greater number of carpal ligament cell strains tested.

Secretion of proMMP-2, a gelatinase, was detected in lattices under stress (before release), whereas both pro and active forms were detected in conditioned media derived from actively contracting lattices. These results suggest that MMP-2 activation occurred concomitantly with lattice contraction supporting an important role for MMP-2 in processing and remodeling of the collagen lattice by Dupuytren fibroblasts. Elevated levels of active MMP-2 have also been observed in actively remodeling palmar aponeurosis of Dupuytren patients^{31,32}; specifically, nodules and cords exhibited the highest MMP-2 levels compared with normal palmar fascia.³¹ In this study, ilomastat treatment inhibited activity of MMP-2 in all fibroblast cell strains. MMP-1 is involved in proteolytic cleavage of the triple helical structure of type I collagen, exposing fragments that are susceptible to gelatinases including MMP-2. The importance of MMP-2 in mediating lattice contraction and remodeling by oral mucosal and dermal fibroblasts has been demonstrated in previous studies.^{23,33} Both MMP-2 and MMP-9, another gelatinase, have been shown to be upregulated by tendon fibroblasts in response to attachment to collagen type I.³⁴ However, MMP-9 was not detected in our study suggesting that MMP-2 is the dominant gelatinase involved in mediating lattice contraction by palmar fascial fibroblasts.

MT1-MMP, a membrane-bound MMP known to mediate activation of proMMP-2, was expressed in contracting lattices.^{35,36} Exposure of Dupuytren fibroblasts to ilomastat resulted in an upregulation of MT1-MMP protein expression (Fig. 5) and enzymic activity (Fig. 7). This may have been the result of a local feedback mechanism—fibroblasts responding to reduced MMP-2 activity by upregulating MT1-MMP expression to increase proMMP-2 activation. These results reinforce the dynamic nature of cell-matrix interactions: even after MMP secretion has occurred, fibroblasts retain the ability to influence their activity in response to matrix signals through membrane-bound enzymes such as MT1-MMP.

MMP activity is upregulated after tissue injury and is thought to play a central role in normal wound repair by facilitating cell movement through the extracellular matrix and subsequent matrix contraction.⁷ Many of the

processes underlying wound healing are also common to Dupuytren's disease. In particular, fibroblast proliferation, collagen deposition, and appearance of myofibroblasts are prominent features of both granulation tissue and Dupuytren tissue.^{22,25,37} Similarly, fibroblast migration and subsequent matrix contraction are likely to be fundamental to contracture development in Dupuytren's disease as well as wound healing.

Our hypothesis suggests an increase in tissue-level MMP activity is fundamental to contracture development by providing an environment conducive to matrix shortening through increased matrix turnover and remodeling. An increase in MMP activity has similarly been associated with pathologic scarring.³⁸ A further possibility beyond the scope of this current study is that related metalloproteinases such as ADAMs (a disintegrin and metalloproteinase) may also play a key role in lattice contraction *in vitro* and contracture *in vivo*. ADAMs are known to be inhibited by broad-spectrum MMP inhibitors such as ilomastat and participate in cell-matrix interactions.³⁹ The intricate balance between the enzymes that break down and remodel the matrix such as MMPs and ADAMs as well as their respective native inhibitors is undoubtedly a key issue in connective tissue disorders and merits further investigation.

In our study, inhibition of MMP-1 and MMP-2 activity by ilomastat accompanied a reduction in lattice contraction mediated by Dupuytren fibroblasts. The results suggest an important role for MMP activity in matrix processing and organization by Dupuytren fibroblasts *in vitro* and suggest that inhibition of MMP activity may well reduce contracture *in vivo* by reducing fibroblast-mediated matrix contraction.

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