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THE ROLE OF AN MMP INHIBITOR IN THE REGULATION OF MECHANICAL TENSION BY DUPUYTREN'S DISEASE FIBROBLASTS

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Mechanical tension and contracture are two related facets of tissue biology. This study assessed the effect of ilomastat, a broad-spectrum matrix metalloprotease (MMP) inhibitor, on generation of tension by Dupuytren's disease fibroblasts. Nodule and cord-derived fibroblasts were isolated from five patients with Dupuytren's disease; flexor retinaculum acted as the control. A culture force monitor (CFM) provided an *in vitro* model of tissue organization to assess development of mechanical tension, lattice contraction and spatial remodelling by fibroblasts. Responses to ilomastat were compared to treatment with a control peptide. Nodule and cord-derived fibroblasts exhibited a two-fold increase in tension compared with flexor retinaculum. Ilomastat significantly inhibited development of tension by nodule and cord but not flexor retinaculum derived fibroblasts at 100 μM. These results imply that MMP activity mediates regulation of tensile strength by Dupuytren's disease fibroblasts and may be an important therapeutic target in patients with Dupuytren's disease.

Keywords: Dupuytren's disease, MMP, ilomastat, tension, fibroblast, matrix remodelling

INTRODUCTION

Dupuytren's disease (DD) is a common fibroproliferative condition of the palmar fascia of the hand that causes disability through progressive digital contracture. Despite advances in surgical management, recurrent contracture remains a common complication (Dias and Braybrooke, 2006), posing a technical challenge and often resulting in poorer functional outcomes. However, in the absence of an effective non-operative alternative treatment, surgery remains the cornerstone of management.

The MMPs are a group of zinc-dependent endopeptidases that cleave extracellular matrix (ECM) components and participate in many biological processes (Sternlicht and Werb, 2001). Through matrix resorption, MMPs facilitate tissue remodelling and cell migration (Nagase et al., 2006), thereby influencing the development of mechanical tension. Tension is intricately linked to wound healing and is an important factor in guiding incision placement and method of wound closure in clinical practice. It has also been identified as a possible contributing factor to DD pathogenesis (Citron and Hearnden, 2003).

MMPs are usually present at low levels and their expression is tightly regulated by growth factors and cytokines including the TIMPs (tissue inhibitors of metalloproteinases), an important family of endogenous inhibitors. Loss of control of MMP activity is implicated in numerous pathological conditions where scarring is prominent including glaucoma and atherosclerosis (Sheridan et al., 2001; Tayebjee et al., 2005). There is also an accumulating body of evidence to suggest that abnormal MMP activity is a feature of DD (Augoff et al., 2006; Johnston et al., 2007), yielding a fresh lead in the search for non-operative therapies.

The culture force monitor (CFM) is an *in vitro* kinetic model that reproduces cell-mediated tissue organization. The CFM enables precise quantification of the generation of mechanical tension through fibroblast-mediated matrix contraction and remodelling (Eastwood et al., 1994), two processes that are thought to underlie development of permanent tissue contracture *in vivo* (Tomasek et al., 2002). Our previous work has demonstrated that MMP activity plays a critical role in matrix contraction in *vitro* (Townley et al., 2008). The present study was undertaken to quantify the effect of ilomastat, a broad-spectrum MMP inhibitor, on mechanical tension developed by DD fibroblasts using the CFM model.

METHODS

Cell culture

Five sets of paired DD cord and nodule fibroblasts were isolated by explant culture from excised specimens at elective primary surgical fasciectomy following local

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ethical committee approval. Nodule and cord tissue were distinguished clinically and by histological evaluation (Bisson et al., 2003). In line with previous papers from our group, flexor retinaculum (called carpal ligament in the previous work) was selected as the control tissue and excised at routine carpal tunnel decompression from patients with no clinical evidence of DD (Bisson et al., 2004; Townley et al., 2008). Flexor retinaculum shares some properties with palmar fascia, being a static fascial structure populated by fibroblasts and a few myofibroblasts (Bisson et al., 2003). Cells were maintained in normal growth medium (NGM) at 37°C with 5% CO_2. NGM consisted of Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Paisley, UK) supplemented with Glutamax 2 mM, 10% (vol/vol) foetal calf serum (FCS), penicillin (100 U/ml), and streptomycin (100 μg/ml, all Gibco, UK). Only cells between passages 3 and 5 were used for the experiments.

Ilomastat (GM6001; Calbiochem, UK) and the control peptide (GM6001 negative control, Calbiochem, UK) were prepared from stock solutions diluted in dimethyl sulfoxide (DMSO, Sigma Chemical Co., Dorset, UK). Throughout this study, ilomastat and control peptide were used at 100 μM, as our previous work has shown that MMP activity is inhibited yet fibroblasts remain viable at this concentration (Townley et al., 2008).

Culture force monitor (CFM)

Three-dimensional type I collagen (Sigma, UK) lattices were prepared with 5×10^6 fibroblasts/lattice using a technique described previously (Bisson et al., 2004). The resultant fibroblast–collagen suspension was dispensed into a sterile rectangular well of a PTFE mould between two flotation bars (Fig 1) and placed in an incubator at 37°C to allow lattice polymerization.

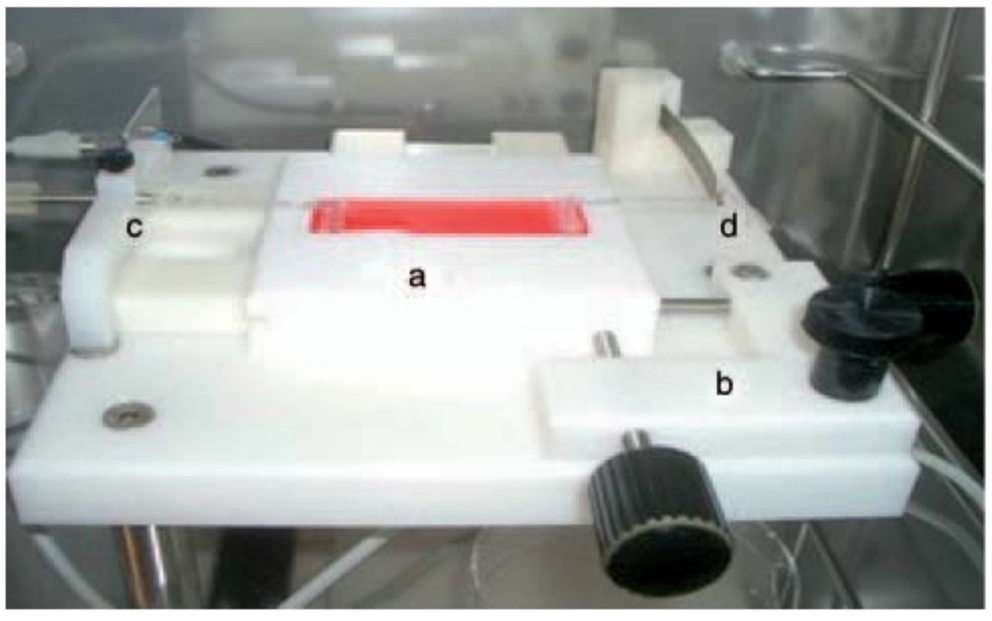

Fig 1 Illustration of the culture force monitor positioned in the incubator. The PTFE mould (a) is positioned on a platform with a moveable mounting stage (b). One flotation bar is attached to a fixed strut on the platform (c), whilst the other is attached to the strain gauge (d).

Effect of ilomastat on force generation

After polymerization, the lattice was released from the well using a sterile needle and 20 ml of test media (NGM ± 100 μM ilomastat or control peptide) dispensed into the well. The mould was transferred to the culture force monitor platform situated within a humidified incubator (37°C and 5% CO_2). One flotation bar was attached to a fixed strut of the platform and the other was hooked onto a force transducer (calibrated in dynes). The lattice was allowed to contract for 48 hours, producing a contraction profile over time for each cell line and experimental condition investigated. The force at equilibrium was set at zero and subsequent readings were recorded every 800 ms by a computer software programme (Chart Recorder, Gray Institute, Northwood, UK).

Effect of ilomastat on matrix remodelling by addition of cytochalasin-D

Cytochalasin-D was used to block the actin-dependent processes, allowing the uncoupling of force generation by fibroblasts through contraction (actin-dependent) and spatial remodelling (actin-independent). After allowing lattices to contract for 48 hours a single dose of cytochalasin-D (Sigma, Poole, Dorset, UK), 20 μl of 60 mM in 0.5 ml of normal growth media, was added to the cell-conditioned test media giving an overall concentration of 60 μM within the chamber. Readings were recorded for a further 2 hours until the force had reached a plateau, revealing a residual force termed residual matrix tension (RMT). A graph was plotted of force against time yielding maximum force generated as well as force attributable to spatial reorganization of the lattice (RMT).

A comparison was made between force generated by DD (paired cord and nodule, $n = 5$) and control fibroblasts (flexor retinaculum, $n = 5$) under basal conditions (NGM) and in response to treatment with ilomastat (100 μM) or control peptide (100 μM).

Statistical analysis

Comparisons between treatments were made using either a two-tailed Student's *t*-test for comparisons between two groups or a one-way ANOVA for multiple groups. The potential for a cumulative type 1 error was deemed to be minimal as two of the three variable conditions were controls and there was no post-hoc statistical analysis. Probabilities with $P < 0.05$ were therefore considered statistically significant. The data are described in terms of mean and standard error of the means.

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RESULTS

Effect of ilomastat on ability of fibroblasts to generate force

The mean contraction profiles of fibroblasts in response to GFM, ilomastat or control peptide are illustrated in Fig 2. Development of tension by all three cell types appeared to be maximal at 48 hours although the actual magnitude of force varied depending on fibroblast origin. Exposure to ilomastat (100 μM) resulted in a significant suppression in the ability of DD cord and nodule-derived fibroblasts to generate force ($P<0.05$) compared to basal conditions (GFM). For cord, the differences were statistically apparent at 42 and 48 hours, whereas for nodule, a significant difference was first observed at 12 hours ($P=0.03$) and maintained until addition of cytochalasin-D at 48 hours. Exposure to ilomastat similarly appeared to reduce force generation by flexor retinaculum-derived fibroblasts although the difference never reached significance over the time course. For all three fibroblast types, GFM-mediated contraction did not appear to be significantly different from exposure to control peptide.

A comparison of the maximum tension generated by different fibroblast types in response to ilomastat and control conditions is illustrated in Fig 3. Nodule-derived fibroblasts appeared to generate greater levels of force (176 [15] dynes) than cord (143 [18] dynes) under basal conditions (GFM) although the difference did not reach significance. By contrast, all DD fibroblasts demonstrated greater force development than flexor retinaculum (72 [10] dynes, $P=0.001$). Treatment with ilomastat significantly inhibited force development by cord (71 [10] dynes, $P<0.05$) and nodule (80 [8] dynes, $P<0.001$) but not flexor retinaculum (51 [10] dynes, $P=0.16$).

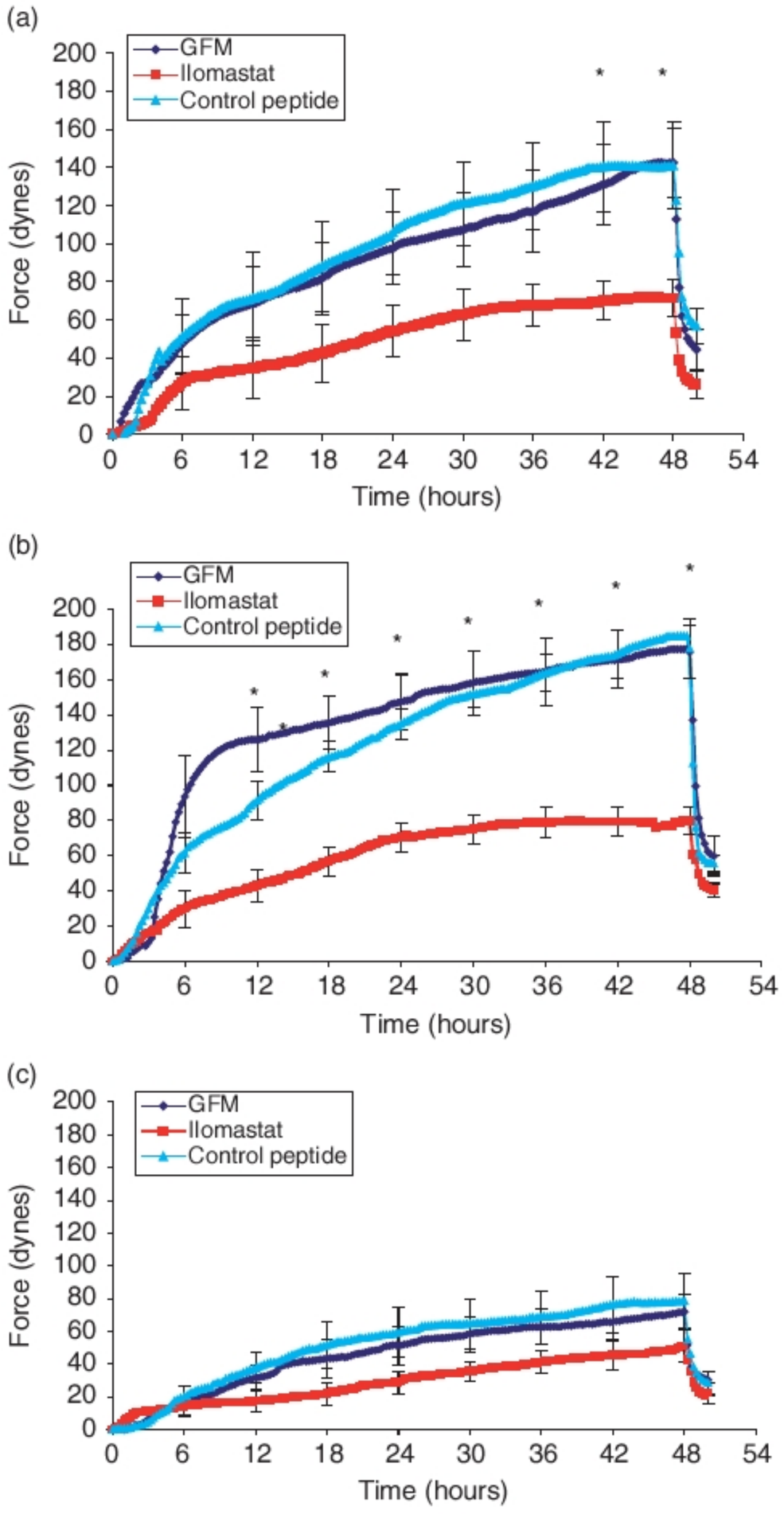

Fig 2 Illustration of force generation by fibroblasts ($n=5$) derived from cord (A), nodule (B) and flexor retinaculum (C). Cytochalasin-D was added to the system after 48 hours. *$P<0.05$ represents a significant difference between ilomastat exposure and control conditions (GFM or control peptide). Mean (SEM).

Effect of ilomastat on residual matrix tension

On addition of cytochalasin-D, the level of force generated by all three fibroblast types reduced sharply (Fig 4.), revealing a RMT of 44 (11) dynes for cord-derived fibroblasts, 60 (11) dynes for nodule and 35 (5)

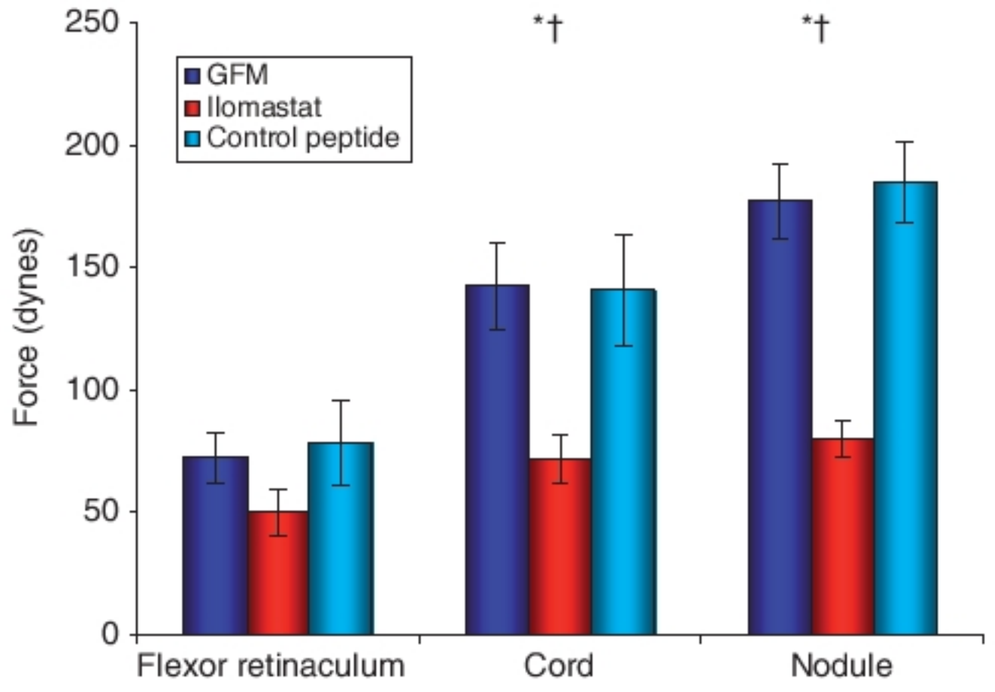

Fig 3 Maximum force generated by flexor retinaculum ($n=5$), cord ($n=5$) and nodule-derived fibroblasts ($n=5$) in response to GFM, ilomastat or control peptide. *$P<0.05$ represents a significant difference between ilomastat and GFM or control peptide. †$P<0.01$ represents a significant difference in response to GFM between DD and flexor retinaculum fibroblasts. Mean (SEM).

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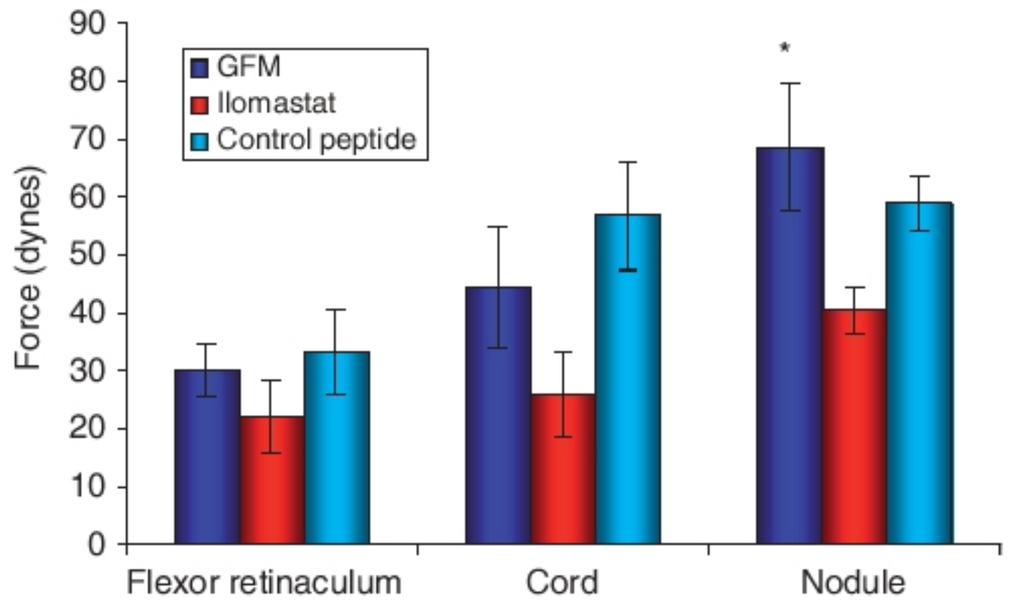

Fig 4 Histogram demonstrating the residual matrix tension of DD and control lattices ($n=5$). Fibroblasts were exposed to either GFM, ilomastat or control peptide. *$P<0.05$ represents a significant increase of nodule over flexor retinaculum in response to GFM. Mean (SEM).

dynes for flexor retinaculum, under basal conditions (GFM). The RMT value for nodule, but not cord, was significantly greater ($P<0.05$) than flexor retinaculum suggesting that nodule-derived fibroblasts were able to remodel lattices to a greater extent than control cells. Exposure to ilomastat reduced absolute RMT values for all three fibroblast types (26 [7], 40 [4], 22 [6] dynes, respectively) suggesting an impact of MMP activity on the ability of fibroblasts to spatially remodel the collagen lattice. However, in each fibroblast group, the difference in RMT between ilomastat and control conditions did not achieve statistical significance.

DISCUSSION

Mechanical tension and tissue contracture are two related facets of tissue biology that are central to Dupuytren's disease. This study addressed the relationship between generation of tension by DD fibroblasts and MMP activity. The culture force monitor was selected to investigate contractile processes as it provides actual kinetic information on force generation by fibroblasts embedded in a collagen matrix (Eastwood et al., 1994). Furthermore, in the CFM model, force generated by contracting fibroblasts is transferred into a force maintained by permanent matrix shortening (Marenzana et al., 2006). This latter process requires spatial reorganization of the matrix, or matrix remodelling, to achieve actual shortening, which is thought to be fundamental to development of contracture (Tomasek et al., 2002).

Ilomastat is a broad-spectrum inhibitor that has been demonstrated to inhibit matrix contraction by Dupuytren's fibroblasts *in vitro* (Townley et al., 2008), reduce scarring in an experimental model of glaucoma filtration surgery (Wong et al., 2003) and prevent human lens capsular contracture (Daniels et al., 2003).

In our model, nodule and cord-derived fibroblasts generated significantly greater tension than control cells under basal conditions. The finding of increased contractility of DD tissue compared to control palmar fascia has been demonstrated previously (Howard et al., 2003), suggesting that the difference was not simply attributable to selection of flexor retinaculum as the control tissue. Exposure to ilomastat significantly inhibited the maximum force obtained by DD fibroblasts (both cord and nodule), suggesting that MMP activity influences the ability of fibroblasts to generate contractile force. However, ilomastat-mediated suppression of MMP activity did not significantly affect tension development by flexor retinaculum derived fibroblasts. The findings suggest a role for MMP activity in regulation of tensile force in diseased but not normal palmar fascia.

Previous work using control dermal fibroblasts similarly failed to demonstrate an effect of ilomastat on development of tension in the CFM model (Phillips et al., 2003), underlining the notion that MMP activity is not uniformly critical to force development, but important in diseased tissue, where it may facilitate contracture development. Indeed, evidence suggests that MMP gene expression is abnormal in DD tissue compared to control tissue (Johnston et al., 2007; Qian et al., 2004) and clinically, increased expression of several MMPs has been shown to correlate with a poor outcome following primary surgery (Johnston et al., 2008). A detailed account of the pattern of ilomastat-mediated suppression of MMP activity in DD fibroblasts was undertaken but mirrored previous findings and was therefore not duplicated here (Townley et al., 2008).

A key interest in our investigation was to establish how MMP activity influences the transfer of force generated by contracting fibroblasts to force retained in the collagen lattice. In absolute terms, exposure to ilomastat reduced the RMT of both disease and control fibroblasts, but the difference in each case did not reach statistical significance. The pattern suggested MMP activity is an important mediator of spatial remodelling of the matrix but did not provide conclusive proof. Spatial remodelling is a time-dependent process and ilomastat may have been shown to influence the remodelling ability of DD fibroblasts over greater time courses. However, the experimental duration was restricted to 48 hours to prevent infection and avoid the need for growth medium changes, which affect the force output reading in the CFM system.

The link between tension and lattice contraction is well established *in vitro* (Marenzana et al., 2006; Mudera et al., 2000). Specifically, generation of tensile force is thought to occur initially through fibroblast migration (Ehrlich and Rajaratnam, 1990; Harris et al., 1981; Tranquillo et al., 1999) and subsequently by myofibroblast-mediated contraction (Gabbiani and Majno, 1972; Hueston et al., 1976). *In vivo*, the presence of myofibroblasts is associated with tissue contracture in disease processes such as hypertrophic scarring, where

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mechanical tension is thought to play a critical role (Aarabi et al., 2007; Ehrlich et al., 1994). Furthermore, mechanical load may influence the development of contracture in DD disease, as supported by clinical evidence suggesting that tension-relieving incisions at primary surgery can reduce recurrence (Citron and Hearnden, 2003).

In this study, we have demonstrated that fibroblasts derived from cord and nodule tissue develop greater mechanical tension *in vitro* compared with control flexor retinaculum. Ilomastat-mediated suppression of MMP activity resulted in a reduction in the development of force in the CFM system suggesting that MMP activity may be an important therapeutic target in patients with Dupuytren's contracture. However, it must be acknowledged that modulation of MMP activity may not provide the whole solution. Further investigation of inhibitors such as TIMPs and other important metalloproteinases may help identify putative therapeutic agents with an appropriate selectivity profile to target disease processes and diseased tissue effectively.

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CONFLICT OF INTERESTS

None declared.

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