HAND (2011) 6:159–164
DOI 10.1007/s11552-010-9314-4

ORIGINAL ARTICLE

Calcium-dependent signaling in Dupuytren's disease

Josef G. Hadeed · Jennifer E. Bond · M. Angelica Selim · Andrew Bergeron · L. Scott Levin · Howard Levinson

Received: 1 September 2010 / Accepted: 29 November 2010 / Published online: 15 December 2010

Abstract
Background Previous studies suggest that Dupuytren's disease is caused by fibroblast and myofibroblast contractility. Cell contractility in smooth muscle cells is caused by calcium-dependent and calcium-independent signaling mechanisms. In the calcium-dependent pathway, calcium/calmodulin activates myosin light chain kinase (MLCK). In this study, the effects of calcium/calmodulin inhibition with the FDA-approved drug fluphenazine on Dupuytren's fibroblast contractility and MLCK expression were tested.
Methods Fibroblast lines from the palmar fascia of patients with Dupuytren's disease were explanted and used for in vitro study. The effect of fluphenazine on Dupuytren's fibroblast migration was determined using a scratch migration assay, and contractility was determined using fibroblast-populated collagen lattice (FPCL) assays. Immunohistochemical staining of MLCK in different samples of Dupuytren's tissue and normal fascia were compared.
Results Fluphenazine demonstrated a dose-dependent inhibition of Dupuytren's fibroblast migration, with the maximum inhibition of migration observed at 20 μM (69.8±1.9%). Fluphenazine also inhibited FPCL contraction in a dose-dependent manner. Maximal inhibition was observed at a fluphenazine concentration of 20 μM (52.5±6.1%). Immunohistological staining illustrated that MLCK was predominantly expressed throughout the cytoplasm of select fibroblasts within Dupuytren's nodules, yet was absent in the fibroblasts of Dupuytren's cords and normal palmar fascia.
Conclusions Fluphenazine inhibits Dupuytren's fibroblast contractility and migration through inhibition of MLCK in vitro. However, the inconsistent expression of MLCK throughout Dupuytren's tissue suggests that calcium-dependent signaling may not be a primary mode of contracture formation. Fluphenazine inhibition of MLCK is not likely to be a target for the treatment of Dupuytren's disease.

Keywords Calcium-dependent signaling

J. G. Hadeed · J. E. Bond · A. Bergeron · H. Levinson (✉)
Division of Plastic, Reconstructive, Maxillofacial and Oral Surgery, Department of Surgery, Duke University Medical Center, 3181,
Durham, NC 27710, USA
e-mail: howard.levinson@duke.edu

M. A. Selim · H. Levinson
Department of Pathology, Duke University Medical Center, 3712,
Durham, NC 27710, USA

L. S. Levin
Department of Orthopedic Surgery,
Hospital of the University of Pennsylvania,
2 Silverstein, 3400 Spruce Street,
Philadelphia, PA 19104, USA

Dupuytren's disease is an idiopathic, prevalent fibrocontractile disease that can be severely debilitating to hand function. Diseased fascia contains nodules that are believed to be the primary epicenter of disease progression because they frequently precede cord formation and contain highly contractile myofibroblasts [33]. Increased fibroblast and myofibroblast migration and contractility were thought to cause Dupuytren's disease. Fibroblast and myofibroblast migration and contraction result from a complicated series of interacting of pathways resulting in the phosphorylation of myosin light chain [21]. Myofibroblasts and fibroblasts are rich in actin and myosin contractile proteins, which are activated by upstream signaling kinases such as calcium-independent Rho associated kinase and calcium-dependent myosin light chain kinase (MLCK) [5]. It is possible that

the increased expression or activity of these signaling kinases could play a role in Dupuytren's contracture. The role of MLCK in Dupuytren's disease has not been investigated.

MLCK is activated by increases in intracellular calcium. Intracellular calcium is increased by extracellular stimuli that promote release from intracellular stores and influx from the extracellular space. Intracellular calcium binds to calmodulin which in turn activates MLCK, resulting in the phosphorylation of the regulatory myosin light chain (MRLC) peptide on myosin II [11, 20, 31, 37]. The increase in MRLC activity produces actin–myosin contraction and, in dermal fibroblasts and smooth muscle cells, increased migration and collagen contraction. This calcium–calmodulin-dependent MLCK activity has not been investigated in Dupuytren's fibroblast activity.

Calmodulin-dependent MLCK activity is inhibited by the Food and Drug Administration (FDA)-approved phenothiazine derivatives, such as fluphenazine [24–26, 43]. In this study, we determined if MLCK is capable of inducing Dupuytren's fibroblast activity by inhibiting calmodulin-dependent MLCK function with fluphenazine and assessed the effect on cell contraction and migration in vitro. This data could suggest calmodulin-dependent MLCK inhibition with fluphenazine is a potential target for preventing Dupuytren's disease. Current treatments are marginally effective and there is a need to discover novel and better therapeutics. By providing new insights into the molecular mechanisms into signaling pathways of Dupuytren's fibroblast activity, more focused Dupuytren treatments can be developed.

Materials and Methods

Human Tissue and Fibroblast Explantation

All tissues were obtained in accordance with the Duke University Medical Center Institutional Review Board. Formalin-embedded Dupuytren tissues for immunohistological staining were obtained from the Duke University Medical Center, Department of Pathology repository of tissue specimens; normal cadaveric palmar fascia was obtained from the Duke Human Fresh Tissue Laboratory and used for immunohistological staining. Dupuytren fibroblasts were explanted from patients undergoing surgical fasciectomy. Dupuytren's nodules were then dissected from the surrounding cords and palmar fascia. In brief, the tissue samples were washed, finely minced, and incubated in collagenase type I in Hank's balanced salt solution with 1% penicillin/streptomycin at 37°C for 4 h. The cells were subsequently cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA). Cells were maintained in a 37°C incubator with 5% CO_2. Experiments with primary cell cultures were performed when cells were 80–90% confluent between passages 1 and 6. For in vitro experiments, four cell strains from different patients were used.

Immunohistochemical Staining

Consecutive sections of 5 μm for each tissue specimen were mounted on silanized charged slides and allowed to dry for 30 min in an incubator at 60°C. After deparaffinization in xylene and rehydration, slides were covered for 10 min with 3% hydrogen peroxide to block endogenous peroxidase. Slides were then placed in citrate antigen retrieval buffer for 20 min at 100°C, followed by washing in Tris-buffered saline Polysorbate-20 (TBST), 0.5 M Tris Base, 9% NaCl, 0.5% Polysorbate-20 (pH 8.4) at room temperature. Slides were then placed in a humidified chamber and incubated for 45 min with mouse anti-MLCK at 1:500 dilution (Clone K36, Sigma-Aldrich. St. Louis, MO). After three rinses in TBST, the slides were incubated for 45 min with biotinylated secondary detection antibodies (horse anti-mouse at 1:200 dilution, Vector, Burlingame, CA). After three rinses in TBST, the slides were incubated with the detection system (Vectastain Elite ABC, Vector Laboratories, Inc., Burlingame, CA) for 30 min. Tissue staining was visualized with a 3,3'-diaminobenzidine substrate chromogen solution (Dako, Carpinteria, CA). Non-immune controls were performed by using a mouse IgG at matched concentration. Sections were analyzed with the assistance by a dermatopathologist.

Scratch Migration Assay

Fibroblasts were added to a 24-well plate at 4.5×10^4 cells per milliliter and allowed to adhere for at least 8 h. The media was drawn off and the wells were scratched with a P-20 pipette tip. The wells were washed with PBS to remove any non-adherent cells. A mark across the bottom of the well, to be used as a reference point, was made perpendicular to the scratch before the cells were added. Fluphenazine (0.63–20 μM in growth media) (Sigma-Aldrich, St. Louis, MO), growth media or DMEM alone was added to the wells. Migration was measured by capturing an image of each well at the reference point at 0, 11, 15, and 19 h. The images were analyzed with ImageJ (NIH, Bethesda, MD) software. All data were repeated for four cell strains in triplicate.

Fibroblast-Populated Collagen Lattices

Fibroblast-populated collagen lattices (FPCL) were made as follows: 400 μL of fibroblast–collagen–medium mixture at

concentration of 1.0×10^5cells per milliliter, 1.28 mg/mL purified collagen (Nutragen, Advanced Biomatrix, Fremont, CA, USA) was added into a 24-well flat bottom tissue culture plate that had been blocked with bovine serum albumin (BSA). The plate was placed in a 37°C incubator with 5% CO_2 for 60 min to allow the collagen to polymerize, before adding 500 μL of 0.5% BSA in DMEM, media or fluphenazine in media (0.63–20 μM). FPCL were freed from the surface of the wells. The sizes of lattices were recorded using a digital scanner at 2, 8, and 10 h. The areas of each lattice were determined and measured using the ImageJ (NIH, Bethesda, MD) software. All conditions were performed in triplicate per experiment, for four cell strains.

Statistical Analysis

All values are presented as mean±standard error of mean. Statistical analysis was performed using JMP software (SAS Institute version 7, Cary, NC). Differences between groups were compared via Student's *t* test and were considered statistically significant at values of $p\leq0.05$.

Results

Immunohistochemical Staining of Dupuytren's Disease and Normal Palmar Fascia

Routine histologic sections of Dupuytren's nodules and cords from four different patients and normal palmar fascia from four different cadavers were stained for MLCK expression. Low-power magnification reveals expression of MLCK in Dupuytren's nodules but little to absent expression of MLCK within Dupuytren's cords and normal palmar fascia (Fig. 1). High-power magnification of the same specimens demonstrates predominant expression of MLCK in the cytoplasm of nodule fibroblasts. Immunohistological staining of the Duputryen tissue indicates that MLCK is more robustly expressed and widely distributed throughout the cytoplasm of Dupuytren's nodule fibroblasts as compared to the scant perinuclear localization seen within Dupuytren's cords fibroblasts and normal palmar fascia fibroblasts.

Effects of Fluphenazine on Fibroblast Activity

Fibroblasts were explanted from tissue excised from four separate patients undergoing surgery for Dupuytren's contracture. The effect of fluphenazine on Dupuytren's fibroblast migration was examined using a scratch assay. In the cell scratch assay, migration was stimulated by FBS; this was the maximum migration rate observed. Both the effect of concentration and exposure time to fluphenazine were evaluated. Fluphenazine demonstrated a dose-dependent inhibition of Dupuytren's fibroblast migration. The maximum inhibition of migration was observed at 20 μM, the highest concentration of fluphenazine assayed, with 69.8±1.9% of Dupuytren's fibroblasts having not migrated (Fig. 2a,b). Fluphenazine inhibited migration compared to FBS that stimulated migration after 11 h. After 15 h, migration of Dupuytren's fibroblasts was only inhibited by 20 μM of fluphenazine as compared to the control.

MLCK-Promoted FPCL Contraction was Blocked by Fluphenazine

Dupuytren's fibroblast was cultured in 3D free-floating FPCLs and contraction measured. Data is reported as a percent of area of the original size of the FPCL, i.e., the smaller the percentage in area the smaller the FPCL and the greater the contraction. Dupuytren's fibroblasts were highly contractile, with the FBS-stimulated contraction decreasing the area of FPCL to 13.7±0.3% after 8 h. Fluphenazine inhibited FPCL contraction in a dose-dependent manner, and a concentration of greater than 2.5 μM was required to significantly inhibit Dupuytren's FPCL contraction ($p\leq$

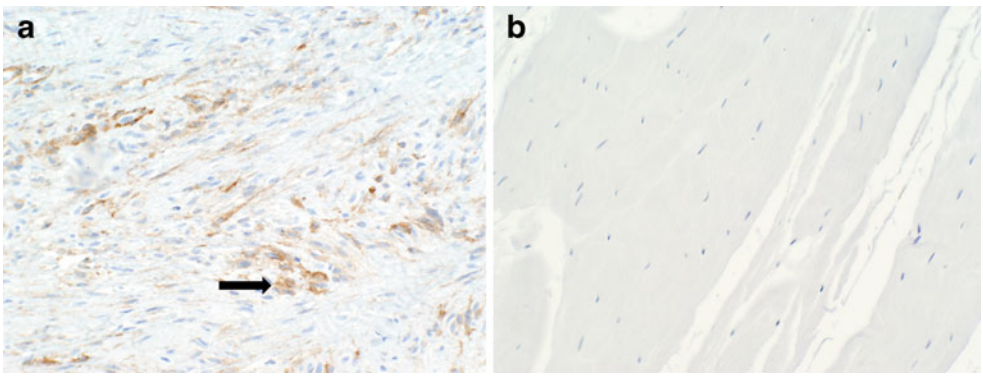

Fig. 1 Expression of MLCK in Dupuytren's tissue. Histologic sections of Dupuytren's nodules of a representative patient. **a** Immunohistochemical staining demonstrates MLCK (*solid arrow*) is focally expressed in select nodules compared to **b** normal palmar fascia ×20 magnification: *scale bar*=100 μm

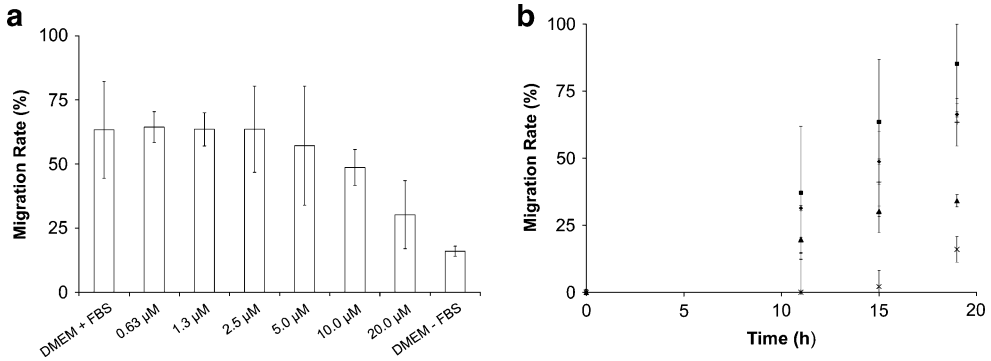

Fig. 2 Inhibition of migration of Dupuytren's fibroblasts by fluphenazine. Fibroblasts were explanted from Dupuytren's tissue and cultured in 24-well plates, following which a scratch was made and fluphenazine was added to the medium at 0–20 μM. Migration of Dupuytren's fibroblasts were measure by taking images at 15 h then measuring the unmigrated area using ImageJ; data is reported as migration rate in percent. **a** Maximum migration rate was observed in DMEM+FBS and the minimum migration rate was observed in DMEM–FBS Dupuytren's fibroblast migration was inhibited by fluphenazine in a dose-dependent manner. **b** The effect of fluphenazine concentration on Dupuytren's fibroblast migration over 20 h DMEM–FBS (*ex mark*), DMEM+FBS (*plus sign*), 20 μM (*black triangle*), 10 μM (*black diamond*), 2.5 μM (*black square*). Data is presented as mean±standard error

0.05; Fig. 3a, b). The maximum inhibition of percentage decrease in Dupuytren's FPCL area (52.5±6.1%) was observed at a concentration of 20 μM of fluphenazine. After 20 h, contraction of Dupuytren's FPCL was only inhibited by concentrations of fluphenazine at 20 μM compared to controls.

Discussion

Dupuytren's disease is an idiopathic, prevalent fibrocontractile disease of the palmar fascia that can be severely debilitating to hand function. There are several comorbid conditions which suggest an increased predisposition for Dupuytren's disease formation, including male gender, increased age, Northern European descent, history of smoking, repetitive trauma to the hands, family history, diabetes, and seizures [4, 6, 13–19, 27, 32, 34, 45].

Diseased palmar fascia contains nodules and cords and although it is unknown why these structures develop, it has been proposed that nodules are the primary epicenter of disease progression because they frequently precede cord formation and contain highly contractile myofibroblasts [33]. While some investigators believe that myofibroblasts are putatively responsible for causing Dupuytren's disease progression, it is interesting to note that not all nodules contain myofibroblasts [9]. Although the pathogenesis of Dupuytren's disease is unknown, it does share similarities to scar contracture [9, 41]. Both occur incrementally as a result of fibroblast and myofibroblast contractility [7, 38].

Actomyosin-based cell contractility throughout the human body has been well studied and the findings may be relevant to Dupuytren's disease [28, 39]. It is uncertain exactly whether myofibroblasts are regulated in a manner similar to that of fibroblasts or to smooth muscle cells, as alpha smooth muscle actin is expressed in stress fibers of differentiated

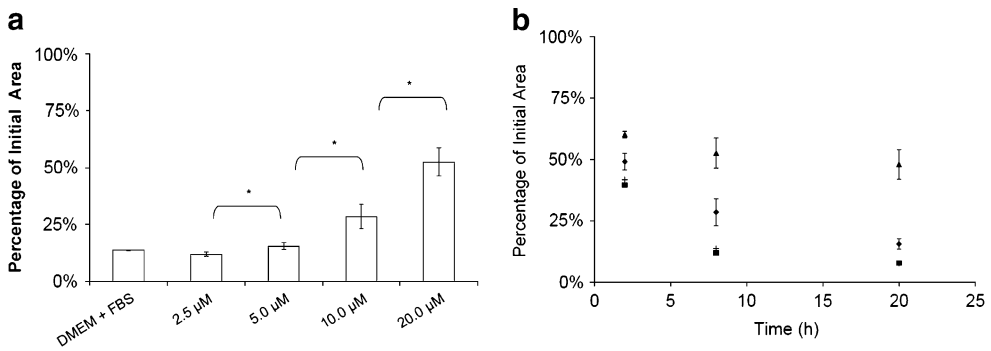

Fig. 3 The effect of fluphenazine on contraction of Dupuytren's fibroblast-populated collagen lattices. Dupuytren's fibroblast was cultured within 3D collagen lattices, contraction of free-floating FPCL was measured by taking images at **a** 8 h and analysis of the area of the FPCL with ImageJ. Fluphenazine inhibited Dupuytren's FPCL contraction. **b** The effect of fluphenazine concentration on Dupuytren's FPCL contraction over 20 h, DMEM+FBS (+), 20 μM (*triangle*), 10 μM (*black diamond*), 2.5 μM (*black square*). Data is presented as mean±standard error

myofibroblasts [7]. There are two systems which appear to regulate the phosphorylation of MRLC, which in turn regulates the contractile stress fibers of myofibroblasts: the calcium-dependent calmodulin–MLCK pathway and the calcium-independent Rho–kinase pathway [5]. It has been demonstrated that the Rho–kinase pathway is the predominant regulatory pathway for myofibroblast contraction [3, 29]. However, little is known regarding the relative contribution of each regulatory pathway to myofibroblast contractility in pathophysiologic states, such as Dupuytren's disease [10]. It is important to elucidate which regulatory pathway of myofibroblast contractility predominates in Dupuytren's disease, as there are currently several FDA-approved drugs that block each pathway [22, 23, 30, 42].

Both regulatory pathways work by activating MRLC. This is done by either directly phosphorylating MRLC at seronine or threonine residues or indirectly by phosphorylating and deactivating the regulatory subunit of MRLC phosphatase [35, 36]. Activated MRLC, in turn, extends the neck domain of the cytoskeletal protein non-muscle myosin II (NMMII). This facilitates actin binding (β-actin in fibroblasts and α-smooth muscle actin and β-actin in myofibroblasts) at the NMMII N-terminal motor domain, thereby conferring ATPase activity. This interaction of β-, γ-, or α-smooth muscle actin with NMMII is believed to mediate cell force generation in Dupuytren's fibroblasts [40]. Importantly, NMMII is believed to be the final common effector protein of all contractile agonists and is therefore hypothesized to be the key regulator of scar contractures and wound contraction. What remains unclear is which of the upstream agonists is most critical in prevention of NMMII expression and subsequent matrix contraction.

When intracellular concentrations of calcium increase, the calcium binds to calmodulin which in turn binds to and activates MLCK [11, 20, 31, 37]. MLCK activity is inhibited by the phenothiazine derivatives class of drugs, commonly used in the treatment of psychosis [24–26, 43]. Inhibition of calcium-dependent myosin light chain peptide phosphorylation has been shown in smooth muscle cells by these agents [44]. Fluphenazine is a member of the phenothiazine class of drugs. It is thought that fluphenazine works by directly binding to the calmodulin–calcium complex, resulting in inhibition of myosin P-light chain phosphorylation [43]. Fluphenazine is used in the treatment of multifocal tic disorders and other psychotic conditions and it is well tolerated [1, 2, 8, 12]. We, therefore, reasoned that its use would be safe in the possible treatment of Dupuytren's disease. We proposed that inhibition of MLCK with fluphenazine would result in reduced fibroblast contractility by blocking the upstream signaling pathway.

Our data shows that fluphenazine inhibits the migration of Dupuytren's fibroblasts in a dose-dependent manner. The greatest amount of inhibition occurred at the highest concentration tested. This most likely is a result of greater saturation of calmodulin receptors at higher doses of fluphenazine. When we looked at the effect of time on fibroblast migration, there was no significant difference noted in the migration of fibroblasts within the first 10 h. After 10 h, however, there was a difference in the amount of cell migration noted. Our studies with unstressed FPCL show that MLCK-mediated fibroblast contractility is mitigated by fluphenazine in a dose-dependent manner, with the maximum inhibition of percentage decrease in Dupuytren's FPCL area observed at the highest concentration of fluphenazine tested. A fluphenazine concentration of greater that 2.5 μM was required to significantly inhibit Dupuytren's FPCL contraction. Immunohistochemical staining of Dupuytren's tissue revealed robust expression of MLCK in Dupuytren's nodules but little to absent expression of MLCK within Dupuytren's cords and normal palmar fascia. This indicates that in Dupuytren's tissue, MLCK is focally located within nodules, but scant overall. From these immunohistochemical findings, it is possible that the calcium-dependent pathway of MLCK activation is not centrally important to progression of Dupuytren's disease. As there are no animal models of Dupuytren's disease, it is difficult to test progression of the disease in vivo.

Scientific discovery of the signaling mechanisms of Dupuytren's contracture formation is the key to developing novel interventions to prevent disease progression. Dupuytren's disease is a slowly progressive fibrocontractile process, and the mechanism of contraction is believed to be similar to those that cause scar contractures. Our data support the notion that MLCK activation may play a small role in disease progression but is unlikely to have a major impact. Future research should look at the role of calcium-independent pathways, such as with Rho–kinase, in disease progression. The long-term goal is to identify the proteins that cause fascia contraction and their location, so that new agonist/antagonistic drugs can be developed or an existing drug can be modified for use in the clinic.

Conclusion

In summary, fluphenazine inhibits Dupuytren's fibroblast contractility and migration through inhibition of MLCK in vitro. The inconsistent expression of MLCK throughout Dupuytren's nodules suggests that contractures may develop from calcium-independent signaling. Fluphenazine inhibition of MLCK alone is not likely to be a target for the treatment of Dupuytren's disease due to inconsistent expression.

Acknowledgements This project was supported by an NIH-mentored Clinical Scientist Award (K08), grant no. GM085562-01; Plastic Surgery Educational Foundation Fellowship; supplemental support from the Division of Plastic, Reconstructive, Maxillofacial and Oral Surgery; and the Departments of Pathology and Surgery at Duke University Medical Center.

References

1. Altamura AC, Sassella F, Santini A, et al. Intramuscular preparations of antipsychotics: uses and relevance in clinical practice. Drugs. 2003;63:493–512.
2. Altamura AC, Whelpton R, Curry SH. Animal model for investigation of fluphenazine kinetics after administration of long-acting esters. Biopharm Drug Dispos. 1979;1:65–72.
3. Anderson S, DiCesare L, Tan I, et al. Rho-mediated assembly of stress fibers is differentially regulated in corneal fibroblasts and myofibroblasts. Exp Cell Res. 2004;298:574–83.
4. Arafa M, Noble J, Royle SG. Dupuytren's and epilepsy revisited. J Hand Surg (Br). 1992;17:221–4.
5. Burridge K, Chrzanowska-Wodnicka M. Focal adhesions, contractility, and signaling. Ann Rev Cell Dev Biol. 1996;12:463–518.
6. Caroli A, Zanasi S, Marcuzzi A, et al. Epidemiological and structural findings supporting the fibromatous origin of dorsal knuckle pads. J Hand Surg (Br). 1991;16:258–62.
7. Darby I, Skalli O, Gabbiani G. Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest. 1990;63:21–9.
8. Davis JM, Leor M, Watanabe MD, et al. Depot antipsychotic drugs, place in therapy. Drugs. 1994;47:741–73.
9. Gabbiani G, Majno G. Dupuytren's contracture: fibroblast contraction? An ultrastructural study. Am J Pathol. 1972;66:131–46.
10. Ganitkevich V, Hasse V, Pfitzer G. Ca^{2+}-dependent and Ca^{2+}-independent regulation of smooth muscle contraction. J Muscle Res Cell Motil. 2002;23:47–52.
11. Gao Y, Ye LH, Kishi H, et al. Myosin light chain kinase as a multifunctional regulatory protein of smooth muscle contraction. IUBMB Life. 2001;51:337–44.
12. Goetz CG, Tanner CM, Klawans HL. Fluphenazine and multifocal tic disorders. Arch Neurol. 1984;41:271–2.
13. Grasso M, Lania C, Blanco S, et al. The natural history of Peyronie's disease. Arch Esp Urol. 2007;60:326–31.
14. Gudmundsson KG, Arngrimsson R, Jonsson T. Dupuytren's disease, alcohol consumption and alcoholism. Scand J Prim Health Care. 2001;19:186–90.
15. Gudmundsson KG, Arngrimsson R, Jonsson T. Eighteen years follow-up study of the clinical manifestations and progression of Dupuytren's disease. Scand J Rheumatol. 2001;30:31–4.
16. Gudmundsson KG, Arngrimsson R, Sigfusson N, et al. Epidemiology of Dupuytren's disease: clinical, serological, and social assessment. The Reykjavik Study. J Clin Epidemiol. 2000;53:291–6.
17. Haedicke GJ, Sturim HS. Plantar fibromatosis: an isolated disease. Plast Reconstr Surg. 1989;83:296–300.
18. Hindocha S, John S, Stanley JK, et al. The heritability of Dupuytren's disease: familial aggregation and its clinical significance. J Hand Surg (Am). 2006;31:204–10.
19. Hindocha S, Stanley JK, Watson S, et al. Dupuytren's diathesis revisited: evaluation of prognostic indicators for risk of disease recurrence. J Hand Surg (Am). 2006;31:1626–34.
20. Kamm KE, Stull JT. Regulation of smooth muscle contractile elements by second messengers. Annu Rev Physiol. 1989;51:299–313.
21. Kamm KE, Stull JT. Dedicated myosin light chain kinases with diverse cellular functions. J Biol Chem. 2000;276:4527–30.
22. Knobloch K, Redeker J, Vogt PM. Antifibrotic medication using a combination of N-acetyl-L-cystein (NAC) and ACE inhibitors can prevent recurrence of Dupuytren's disease. Med Hypotheses. 2009;73:659–61.
23. Kuhn MA, Wang X, Payne WG. Tamoxifen decreases fibroblast function and downregulates $TGF_{\beta 2}$ in Dupuytren's affected palmar fascia. J Surg Res. 2002;103:146–52.
24. Lau YS, Gnegy ME. Effects of lanthanum and trifluoperazine on [^{125}I] calmodulin binding to rat striatal particulates. J Pharmacol Exp Ther. 1980;215:28–34.
25. Levin RM, Weiss B. Inhibition by trifluoperazine of calmodulin-induced activation of ATPase activity of rat erythrocyte. Neuropharmacology. 1980;19:169–74.
26. Levinson H, Moyer KE, Saggers GC, et al. Calmodulin-myosin light chain kinase inhibition changes fibroflast-populated collagen lattice contraction, cell migration, focal adhesion formation, and wound contraction. Wound Repair Regen. 2004;12:505–11.
27. Ling RS. The genetic factor in Dupuytren's disease. J Bone Joint Surg (Br). 1963;45:709–18.
28. Naumanen P, Lappalainen P, Hotulainen P. Mechanisms of actin stress fibre assembly. J Microsc. 2008;231:446–54.
29. Parizi M, Howard EW, Tomasek JJ. Regulation of LPA-promoted myofibroblast contraction: role of Rho, myosin light chain kinase, and myosin light chain phosphatase. Exp Cell Res. 2000;254:210–20.
30. Pavelic SK, Bratulic S, Hock K. Screening of potential prodrugs on cells derived from Dupuytren's disease patients. Biomed Pharmacother. 2009;63:577–85.
31. Pfitzer G. Invited review: regulation of myosin phosphorylation in smooth muscle. J Appl Physiol. 2001;91:497–503.
32. Rayan GM. Dupuytren's disease: anatomy, pathology, presentation, and treatment. Instr Course Lect. 2007;56:101–11.
33. Rayan GM, Tomasek JJ. Generation of contractile force by cultured Dupuytren's disease and normal palmar fibroblasts. Tissue Cell. 1994;26(5):747–56.
34. Ross DC. Epidemiology of Dupuytren's disease. Hand Clin. 1999;15:53–62.
35. Sandquist JC, Swenson KI, Demali KA, et al. Rho kinase differentially regulates phosphorylation of nonmuscle myosin II isoforms A and B during cell rounding and migration. J Biol Chem. 2006;281(47):35873–83.
36. Sellers JR, Wang F, Chantler PD. Trifluoperazine inhibits the MgATPase activity and in vitro motility of conventional and unconventional myosins. J Muscle Res Cell Motil. 2003;24(8):579–85.
37. Stull JT, Lin PJ, Krueger JK. Myosin light chain kinase: functional domains and structural motifs. Acta Physiol Scand. 1998;164:471–82.
38. Tomasek JJ, Gabbiani G, Hinz B, et al. Myofibroblasts and mechano-regulation of connective tissue remodeling. Nat Rev Mol Cell Biol. 2002;3:349–63.
39. Tomasek JJ, Haakama CJ, Eddy RJ, et al. Fibroblast contraction occurs on release of tension in attached collagen lattices: dependency on an organized actin cytoskeleton and serum. Anat Rec. 1992;232:359–68.
40. Tomasek J, Rayan GM. Correlation of alpha-smooth muscle actin expression and contraction in Dupuytren's disease fibroblasts. J Hand Surg (Am). 1995;20(3):450–5.
41. Tomasek JJ, Vaughan MB, Haaksma CJ. Cellular structure and biology of Dupuytren's disease. Hand Clin. 1999;15:21–34.
42. Townley WA, Cambrey AD, Khaw PT. Matrix metalloproteinase inhibition reduces contraction by Dupuytren fibroblasts. J Hand Surg Am. 2008;33:1608–16.
43. Vincenzi FF. Calmodulin pharmacology. Cell Calcium. 1981;2:387–409.
44. Weiss B, Levin RM. Mechanism for selectively inhibiting the activation of cyclic nucleotide phophodiesterase and adenylate cyclase by antipsychotic agents. Adv Cyclic Nucleotide Res. 1978;9:285–303.
45. Zachariae L. Dupuytren's contracture. The aetiological role of trauma. Scand J Plast Reconstr Surg. 1971;5:116–9.