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Research Paper

Anti-Tumour Necrosis Factor Therapy for Dupuytren's Disease: A Randomised Dose Response Proof of Concept Phase 2a Clinical Trial

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ABSTRACT

Background: Dupuytren's disease is a common fibrotic condition of the hand that causes irreversible flexion contractures of the fingers, with no approved therapy for early stage disease. Our previous analysis of surgically-excised tissue defined tumour necrosis factor (TNF) as a potential therapeutic target. Here we assessed the efficacy of injecting nodules of Dupuytren's disease with a TNF inhibitor.

Methods: Patients were randomised to receive adalimumab on one occasion in dose cohorts of 15 mg in 0.3 ml, 35 mg in 0.7 ml, or 40 mg in 0.4 ml, or an equivalent volume of placebo in a 3:1 ratio. Two weeks later the injected tissue was surgically excised and analysed. The primary outcome measure was levels of mRNA expression for α-smooth muscle actin (*ACTA2*). Secondary outcomes included levels of α-SMA and collagen proteins. The trial was registered with ClinicalTrial.gov (NCT03180957) and the EudraCT (2015-001780-40).

Findings: We recruited 28 patients, 8 assigned to the 15 mg, 12 to the 35 mg and 8 to the 40 mg adalimumab cohorts. There was no change in mRNA levels for *ACTA2*, *COL1A1*, *COL3A1* and *CDH11*. Levels of α-SMA protein expression in patients treated with 40 mg adalimumab (1.09 ± 0.09 ng per μg of total protein) were significantly lower ($p = 0.006$) compared to placebo treated patients (1.51 ± 0.09 ng/μg). The levels of procollagen type I protein expression were also significantly lower ($p < 0.019$) in the sub group treated with 40 mg adalimumab (474 ± 84 pg/μg total protein) compared with placebo (817 ± 78 pg/μg). There were two serious adverse events, both considered unrelated to the study drug.

Interpretation: In this dose-ranging study, injection of 40 mg of adalimumab in 0.4 ml resulted in down regulation of the myofibroblast phenotype as evidenced by reduction in expression of α-SMA and type I procollagen proteins at 2 weeks. These data form the basis of an ongoing phase 2b clinical trial assessing the efficacy of intranodular injection of 40 mg adalimumab in 0.4 ml compared to an equivalent volume of placebo in patients with early stage Dupuytren's disease.

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1. Introduction

Dupuytren's disease (DD) is a common fibrotic disease confined to the hand that affects approximately 4% of the general UK and US populations [1]. The early stages of the disease are manifest as nodules that are typically quiescent for a period and then become active, progressing to cords and flexion deformities of the fingers in approximately 50% of patients [2] and result in loss of hand function [3]. Whilst the mainstay of treatment remains surgical excision (fasciectomy) of the diseased tissue or cords [4], approximately 40% of patients in the USA are treated by disruption of the cords using collagenase or needle fasciotomy [5]. Generally patients undergo these treatments when digits are flexed to 30° or more and hand function is impaired [6]. The recurrence rate following surgery is 21% within 5 years [7] and these individuals may require more extensive surgery involving excision of the diseased tissue and overlying skin (dermofasciectomy). Post-operatively, some patients require prolonged hand therapy and splintage. Complications occur in approximately 20% of patients undergoing surgery [8]. Alternative, less invasive techniques to disrupt the cords with a needle or collagenase digestion are associated with rapid recovery of hand function with minimal therapy. However, recurrence rates are high, affecting

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85% of patients treated with percutaneous needle aponeurotomy [7] and 32% of those treated with collagenase [9] at 5 years. The complication rate is 20% following needle aponeurotomy [8] and over 70% after collagenase injection, the majority being minor and mostly transient [10].

The ideal therapy would be directed towards patients with early stage disease to prevent progression to development of cords and subsequent flexion contractures of the digits. Our systematic review [11] highlighted the lack of robust evidence for treatments proposed for early stage DD for which there is currently no approved therapy. Studies reporting the efficacy of intranodular injection of steroids or radiotherapy are limited by a lack of quality, with no blinding or randomisation and the use of subjective outcome measures [11]. Fifty percent of patients receiving steroid therapy developed transient subcutaneous atrophy or depigmentation. Approximately 20–30% of patients receiving radiotherapy developed long-term adverse effects, including dry skin, desquamation, skin atrophy, telangiectasia, erythema, and altered heat and pain sensation. A recent trial reported that injection of collagenase resulted in nodules becoming smaller and softer, with approximately 50% of patients experiencing bruising and pain [12]. Therefore, there is a need to develop an effective therapy to retard progression of early DD and also prevent the development of recurrent disease following surgery, needle fasciotomy or collagenase injection in patients with established finger contractures.

The cell responsible for deposition of the excessive collagenous matrix and contraction in all fibrotic conditions, including DD, is the myofibroblast [13, 14], which is characterised by the expression of α-smooth muscle actin (α-SMA) [15]. Unlike the fibrotic diseases of visceral organs such as the kidney, lung and liver, tissue from patients with DD is readily accessible, allowing identification of potential novel therapeutic targets [16]. Using surgically excised tissue from patients we found that the myofibroblasts in DD are aggregated in nodules in the vicinity of the affected joints, and nodules were absent in patients with more advanced stage disease [17]. Interspersed through the nodule are immune cells, including macrophages, T cells and mast cells, and the nodular cells secrete a variety of cytokines [18]. Comparison of the effects of each of these cytokines showed that only tumour necrosis factor (TNF) converted palmar fibroblasts from patients with DD into myofibroblasts at the low concentrations seen *ex vivo,* but not non-palmar fibroblasts from the same patients or palmar fibroblasts from normal individuals. In contrast, TGF-β indiscriminately converts all fibroblasts into myofibroblasts [18]. This is important as the fibrotic process seen in DD is limited to the palm of genetically susceptible individuals. Genome-wide association studies have highlighted the role of Wnt signalling in DD [19, 20] and we found that TNF acted via the Wnt signalling pathway only in palmar dermal fibroblasts from patients with DD [18]. Myofibroblasts from DD showed a dose-dependent reduction in contractility on treatment with anti-TNF, with a concomitant reduction in expression of α-SMA [18]. All the clinically approved anti-TNF agents assessed were effective in down regulating DD myofibroblast contractility *in vitro*, with the two fully human IgG molecules, adalimumab and golimumab being the most efficacious at the doses tested [18].

Here we report the effects of injection of escalating doses of adalimumab or corresponding volume of placebo directly into the nodules of patients who then underwent surgery two weeks later. Markers of myofibroblast phenotype and collagen production were assessed in the excised samples.

2. Methods

2.1. Study Design and Patients

Repurposing anti-TNF for Dupuytren's disease (RIDD) is a two-part phase 2 randomised, double-blinded placebo-controlled dose response study to assess the efficacy of local injection of adalimumab in patients with DD. The protocol was reviewed by the South Central Oxford B Research Ethics Committee (Reference number 15/SC/0259) and the Medicine and Healthcare products Regulatory Authority (EudraCT no: 2015-001780-40) and has been published [21] (details of subsequent amendments in appendix). The phase 2a dose escalation study reported here was performed at a single centre in the UK at the Edinburgh Department of Plastic Surgery at St John's Hospital, NHS Lothian. Patients referred to the hand surgery service at St John's Hospital by their general practitioner with a diagnosis of DD were screened for entry to the trial. Eligibility criteria included no prior treatment for Dupuytren's disease to the affected hand, a clinically distinct nodule of DD, suitability for injection of adalimumab and fasciectomy. All potential participants were screened for TB, HIV, hepatitis B and C using serological testing and chest X-ray in accordance with local standard procedures for anti-TNF screening.

During the study a new preparation of adalimumab became available with 40 mg formulated in 0.4 ml. The lower volume and absence of excipients including citrate was expected to result in reduced pain and improve participant acceptability. Therefore, the trial design was modified to include a cohort at a dose of 40 mg using this preparation. The 40 mg in 0.4 ml formulation is currently only available in a pre-filled single use syringe and so only the full syringe dose of 40 mg could be administered with this formulation. Therefore, the trial design was modified to include a cohort of 40 mg in 0.4 ml. Since publication, the protocol has been amended to introduce an endpoint to assess whether adalimumab affects the healing of the surgical incisions or subsequent scarring. This visual assessment of the surgical wounds was carried out by blinded review of the hand photographs taken at baseline and then at 2 and 4 weeks post-surgery.

2.2. Procedures

Patients were randomised (3:1) to receive either adalimumab or saline in 3 dose cohorts (15 mg in 0.3 ml, 35 mg in 0.7 ml), both using the 40 mg in 0.8 ml preparation, or 40 mg in 0.4 ml using the more recently introduced formulation, or an equivalent volume of placebo (normal saline) on one occasion by intra-nodular injection two weeks (±3 days) prior to scheduled surgery. Nodule hardness was measured using a durometer (RX-1800-00, Rex Gauge Company, Illinois, USA) and nodule size was assessed using an ultrasound scan before injection and again before surgery. Blood was collected at baseline and again immediately before surgery, and the serum stored at -80 °C prior to analysis. ELISA kits from Promonitor were used according to the manufacturer's instructions to measure serum levels of adalimumab (Cat. No. 728552) and anti-adalimumab (Cat. No. 728533). All samples were measured in duplicate and repeated on 3 plates using a FLUOstar Omega Spectrophotometer (BMG Labtech) and MARS™ software. Pain related to the adalimumab injection was rated by the participant and the injection site assessed by visual inspection. Following surgery, the excised Dupuytren's tissue was transported to the lab, where the nodule was dissected to retain a central piece for histological examination whilst the remainder was frozen and pulverised. The resulting powder was split in two for protein or RNA extraction and stored at −80 °C. RNA was extracted and following the generation of cDNA, absolute levels of *ACTA2, COL1A1, COL3A1* and *CDH11* were determined using the standard curve method (appendix). Procollagen 1A1 protein levels were determined by DuoSet ELISA reagents (DY6220-05, R&D systems, Oxon, UK) in triplicate following the manufacturer's instructions. α-SMA protein levels were determined using a custom developed MSD® plate (appendix 2). Tissue samples for histology were fixed in 4% paraformaldehyde, longitudinally bisected, embedded in paraffin wax and 7 μm sections obtained from the cut surface. Sequential sections were stained with hematoxylin-eosin, mouse anti–α-SMA antibody (Abcam 7817) or a mouse isotype control. Antibodies were detected using biotinylated anti-mouse antibody and avidin/biotin/HRP complex reagent (Vectastain ABC, Vector Lab, UK). Patients completed their

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