European Journal of Radiology 82 (2013) e823–e828

Contents lists available at ScienceDirect

European Journal of Radiology

journal homepage: www.elsevier.com/locate/ejrad

Acceleration of tendon healing using US guided intratendinous injection of bevacizumab: First pre-clinical study on a murine model

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ARTICLE INFO

Article history:
Received 22 January 2013
Received in revised form 12 May 2013
Accepted 26 June 2013

Keywords:
Tendon
Anti-angiogenic
Tendinosis
Rat
US

ABSTRACT

Purpose: Tendinopathy shows early disorganized collagen fibers with neo-angiogenesis on histology. Peri-tendinous injection of corticosteroid is the commonly accepted strategy despite the abscence of inflammation in tendinosis. The aim of our study was to assess the potential of intratendinous injection of an anti-angiogenic drug (bevacizumab, AA) to treat tendinopathy in a murine model of patellar and Achilles tendinopathy, and to evaluate its local toxicity.
Materials and method: Forty rats (160 patellar and Achilles tendons) were used for this study. We induced tendinosis (T+) in 80 tendons by injecting under ultrasonography (US) guidance Collagenase 1® (day 0 = D0, patellar = 40 and Achilles = 40). Clinical examination and tendon US were performed at D3, immediately followed by either AA (AAT+, $n = 40$) or physiological serum (PST+, $n = 40$, control) US-guided intratendinous injection. Follow-up at D6 and D13 using clinical, US and histology, and comparison between the 2 groups were performed. To study AA toxicity we compared the 80 remaining normal tendons (T−) after injecting AA in 40 (AAT−).
Results: All AAT+ showed a better joint mobilization compared to PST+ at D6 ($p = 0.004$) with thinner US tendon diameters ($p < 0.004$), and less disorganized collagen fibers and neovessels on histology ($p < 0.05$). There was no difference at D13 regarding clinical status, US tendon diameter and histology ($p > 0.05$). Comparison between AAT− and T− showed no AA toxicity on tendon ($p = 0.18$).
Conclusion: Our study suggests that high dose mono-injection of AA in tendinosis, early after the beginning of the disease, accelerates tendon's healing, with no local toxicity.

1. Introduction

Tendinosis (T+) is a very common and disabling condition, resulting in impairment of quality of life. Indeed, T+ of the rotator cuff affects 3–20% of the general population, mainly women between 40- and 65-year old whereas Achilles' T+ affects 5–6% of the general population, especially young men. In most cases, this condition progresses to a disabling pain or tendon rupture, which can impact, in our experience, personal and professional activities [1,2].

The healthy tendon is made up of type 1 collagen and elastin fibers, within a ground substance containing cells (tenocytes and tenoblastes) and water. In case of T+ (mechanical, traumatic or rheumatic), histology shows thin and disorganized collagen fibers, mucoid and/or lipoïd degeneration and increased inter fibrillar glycosaminoglycan deposition [3,4]. In addition to these lesions, neo-angiogenesis and nerve fibers development have been

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http://dx.doi.org/10.1016/j.ejrad.2013.06.012

reported [5,6], at the beginning of T+ and throughout tendon healing [7–10]. Conversely, inflammatory lesions are rare, but may be associated with tendon rupture [2]. Early treatment of T+ should therefore be recommended to avoid complications.

Several lines of research have been explored for the treatment of T+ and tendon rupture, including ultrasound (US)-guided fenestration or tenotomy [11–14], hyperosmolar solutions [14], bone morphogenic protein [15], or platelet-rich plasma (PRP) intra tendinous injections, with varying efficacy [16]. Despite these potential treatments, peri-tendinous injection of corticosteroid remains the commonly accepted strategy to treat degenerative diseases of the tendon [17] despite the absence of inflammation and the proven serious side effects (tendon rupture) due in part to intra-tendinous injection [18].

Recently, it has been shown that ultrasound-guided sclerosis of neovessels [19] can moderately improve function and reduce pain in a heterogeneous group of patients presenting T+. This therapeutic effect is however limited to the destruction of grown neovessels with no known preventive effect on the development of additional neovessels. Anti-angiogenic therapies (AA) can provide a double therapeutic effect by simultaneously inducing neovessel definitive vasoconstriction and inhibiting growth factors implicated in the development of neovessels. In the cornea, local injection of AA successfully reduces corneal neovascularization [20].

The aim of our study was therefore to assess the potential of intratendinous injection of AA to treat T+ in a rat model of patellar and Achilles T+, and to evaluate its local toxicity.

2. Materials and methods

The procedures and the animal care complied with the "Principles of animal care" formulated by the European Union (Animal Facility Agreement 75-18-03, 2005), and animal experimentation was performed under the authorization 75–101 of the French Ministry of Agriculture.

Forty immunocompetent healthy male Sprague Dawley rats (providing 160 patellar and Achilles tendons, age = 6 weeks old, mean weight = 191 ± 27 g, CERJ, Le Genest, France) were used for the study. The rats were sedated before and during each manipulation with Isoflurane® (5% for induction and 2.5% for maintenance). Two protocols were used, one to assess the efficacy of AA to treat T+ (using 40 patellar and 40 Achilles T+), and a second one to assess AA local toxicity (using 40 patellar and 40 Achilles T+).

During follow-up, rats were housed in groups of 4 in stalling cages at the INSERM U698 conventional animal housing facility. Animals were submitted to a standard laboratory rat pellets with water at libitum, with a 12 h light/dark cycle (12 h light:12 h dark) and a 20 ± 2 °C temperature.

2.1. Protocol 1 (AA efficacy)

At D0, we induced chemical T+ on all 80 patellar and Achilles tendons by a single intra-tendinous injection of type 1 Collagenase Gibco™ (250 U, i.e. 30 μl, dissolved in 0.09% saline solution PROAM®, Honeywell Safety Products) using a 29G needle, under Ultrasonography (US) guidance. This model of T+ has been described in previous publications and permits one to obtain an animal model of T+ as early as 3 days after collagenase injection and thereafter, up to 12 weeks [21,22]. At D3, we assessed T+ using both clinical and US examination, and initiated treatment using either AA or Physiological Serum solution PROAM®, Honeywell Safety Products (PS, control).

Treatment consisted of a single intratendinous injection under US guidance (targeting the thickened segment of the tendon) using a 29G needle of either 0.1 ml (2.5 mg) of AA (AAT+) or 0.1 ml of PS (PST+) [22]. Technically, the needle was inserted in the tendon with an angulation of 45°, and the injection of either collagenase, AA or PS was performed once the needle reached the middle of the tendon. No specific regimen or restricted activity followed the PRP or PS injection.

Among these 80 patellar and Achilles tendons, effectiveness of AA was studied by comparing 40 AAT+ and 40 PST+ at D6 and D13. At D6, clinical and US examination were performed on all 80 tendons with histology on 40 tendons (20 patellar and 20 Achilles tendons). At D13, clinical and, US examination were performed on the 36 remaining tendons (1 rat died between D6 and D13) immediately followed by histology.

We studied the effect of AA and PS 6 and 13 days after T+ induction to obtain data during the early phase of T+ based on the hypothesis that Avastin® (Sanofi™ Laboratory) can rapidly decrease angiogenesis in T+, similar to its effect in corneal neovascularization where improvements can be detected as early as 3 days after local injection of AA [20].

2.2. Protocol 2 (AA toxicity)

AA local toxicity was studied by comparing 80 tendons without chemical T+ induction (T−) injected with AA (AAT−, $n = 40$, patellar = 20, Achilles = 20) or not injected (T−, $n = 40$, patellar = 20, Achilles = 20). Clinical and US T− assessment were performed 3 days and 10 days after AA intra-tendinous injection with histology on every rats at D10.

All left paws are AAT+ in protocol 1 and AAT− in protocol 2. All right paws are PST+ in protocol 1 and T− in protocol 2.

Rats were sacrificed using intra peritoneal injection of 200 ml of Phenobarbital®, Genevrier™ for 100 g of rat.

Clinical evaluation (BD, ML) of each tendon was based on a semi-quantitative clinical score (Score 0: normal mobility, Score 1: moderate mobility of the paw, Score 2: no mobility of the paw). Mobility was assessed by observing during 10 min the spontaneous behavior of rats in their cage.

US examination (BD, ML) was performed using a high frequency ultrasound (US) VEVO 2100 (VisualSonic®) apparatus dedicated to small animals, with a 50 MHz transducer (spatial resolution: 30 μm). We measured tendon antero-posterior diameters (on longitudinal cut) on straight tendons (obtained by dynamic joint flexion), similar to validated human and animal model US tendon measurement methods [22–24]. The transducer was positioned in the visually thickened segment of the tendon. All clinical measurements were performed by two independent operators and recorded after consensus. This US measurement method was previously validated in our institution on a rat model before we initiated our study (unpublished data) with intra-reader variability of 0.05 mm on patellar and Achilles tendon. We calculated an alpha risk of 5% and statistical power of 80% when hypothesizing a difference of 0.1 mm (SD 0.1 mm) of tendon thickness between the AA and the control groups.

Operators were blinded to the status of tendons (either AAT+ or AAT− on the left paw, PST+ or T− on the right paw).

Histology (LD) was performed by fixating tissue samples with formalin for 48 h, embedding in paraffin, and staining 5 μm sections with Hematoxylin and Eosin (HE) and Masson's trichrome (MT). The Bonar score in the T+ targeting area was used in protocol 1 (efficacy study) and in 2 (toxicity study) to assess changes in tendon histology [25].

In addition, we performed immunohistochemistry using ED1 (specific for macrophages in the rat).

2.3. Statistical analyses

Statistical analyzes were performed using MedCalc® software 11.0. To demonstrate the effective induction of T+, we compared