Achilles Tendinopathy is a Troublesome Sports-related Condition Involving Blood Vessel Ingrowth into the Tendon Tissue

Studies on the Adjacent Plantaris Tendon and the Peritendinous Connective Tissue Suggest that TNF-alpha can be Highly Involved in the Vascular and Tissue Changes

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Abstract: Achilles tendinopathy/tendinosis is a troublesome condition which is frequently occurring in response to sports related activities. It can lead to an ending of the sport activity. There is evidence which shows that ingrowth of blood vessels occurs from the peritendinous tissue. In well-established treatments the areas of these vessels are targeted. In Achilles tendinosis there is frequently a coalescing of the plantaris tendon with the Achilles tendon. TNF-alpha is known to be involved in blood vessel remodelling events and angiogenesis. With these facts as background, the peritendinous connective tissue located inbetween the plantaris and Achilles tendons and the plantaris tendon itself in cases with Achilles tendinosis were evaluated concerning expression of TNF-alpha and TNF receptor II (TNFRII). It was found that there were expressions of TNF-alpha in the numerous cells located in the peritendinous connective tissue and that the very frequently occurring blood vessels located in this tissue as well as in the tendon tissue exhibited marked TNFRII reactions. The tenocytes were shown to exhibit moderate TNF-alpha reactions and very strong TNFRII reactions. The observations suggest that TNF-alpha is highly involved in the blood vessel remodelling in tendinosis and that TNF-alpha also is involved in tenocyte function.

1 INTRODUCTION

Midportion Achilles tendinopathy, characterized by chronic Achilles tendon pain, local swelling in the midportion and loss of function (Khan et al., 1999), is very frequent among sports athletes. It is assumed that about 7-9% of professionals performing high frequency of running and jumping suffer from this condition (Cook et al., 2002); (Alfredson, 2003). This makes up 6-18% of all injuries that happen in running disciplines (Alfredson and Lorentzon, 2000); (Fahlström et al., 2002); (Schepsis et al., 2002). It has also been shown that even moderate activity in the form of badminton and track and field activities can lead to the condition (Kvist, 1991); (Fahlström et al., 2002). Repetitive strain is considered to be the main risk factor (Kader et al., 2002); (Paavola et al., 2002) but other aspects like age, sex, training performance, muscle weakness and lack of flexibility seem to be of importance as background factors (Clement et al., 1984); (Haglund-Akerlind and Eriksson, 1993); (Tuite et al., 1997); (Hart et al., 1998); (Dudhia et al., 2007); (Gaida et al., 2010). The precise underlying mechanisms are still unclear.

Achilles tendinopathy is often called Achilles tendinosis when besides pain, swelling and loss of function, structural tissue changes can be observed via ultrasound, MRI or histological evaluation (Khan et al., 1999). A characteristic histological appearance is the occurrence of an increased vascularization; other changes are hypercellularity in early states, decreasing cellularity in later states, cell rounding and decreased matrix organization (Aström et al., 1995); (Alfredson et al., 2003); (Riley, 2008). The blood vessel ingrowth, which occurs from the peritendinous connective tissue, is presumably of great importance. It is thus to the regions with high blood flow, as visualized via colour Doppler coupled with ultrasonography, that treatments frequently are

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directed (Lind et al., 2006); (Alfredson, 2011a).

Currently used treatments for midportion Achilles tendinopathy/tendinosis such as eccentric training, injection treatments and traditional surgical techniques have shown quite good clinical outcome. However, there are still cases that have not been found to be curable (Alfredson, 2011a). Interestingly, it has been shown that 58 out of 73 (80%) Achilles tendinopathy tendons undergoing re-operation with ultrasound+Doppler guided scraping have an invaginated or "close by located" enlarged plantaris tendon (Alfredson, 2011b). During Achilles tendoscopy, it has also been noted that the plantaris tendon can be seen to be affixed to the medial side of the Achilles tendon in cases with tendinopathy (Van Sterkenburg et al., 2011); (Van Sterkenburg and Dijk, 2011).

The peritendinous tissue located outside the Achilles tendon is likely to be of great importance in the situations with tendinopathy and the curing of this. It is thus known that this tissue represents a dynamic and responsive region that markely adapts to exercise (Kjaer, et al., 2000). It is e.g. shown that there is an increase in bradykinin and adenosine concentrations in the peritendinous tissue around the Achilles tendon in response to exercise (Langberg et al., 2002). In comparison, it has in recent studies using a 14C bomb-pulse method been shown that the tendon tissue itself has a poor regenerative capacity, i.e. a lack of tissue renewal (Heinemeier et al., 2013). An important part in the operation procedures when the plantaris tendon is extirpated is a surgical scraping procedure, the scraping being done for the peritendinous connective tissue ventral to the Achilles tendon (Alfredson, 2011c). The scraping is guided by the evaluation of where the high blood occurs, as visualized via ultrasound and laser Doppler (Alfredson, 2011b).

There is a marked presence of peritendinous connective tissue in the region between the plantaris and Achilles tendons. As described above, the two tendons can be very tightly connected via this tissue in situations with Achilles tendinosis/tendinopathy. Almost no attention has been paid to this tissue. The information that exists says that that there is a marked presence of blood vessels in the tissue, but also frequent fibroblasts and to some extent inflammatory cells as well (Spang et al., 2013).

As described above, the peritendinous connective tissue outside tendinopathy tendons may be a very important tissue. It is especially related to the basis for the ingrowth of blood vessels that occurs from this into the tendon tissue in tendinosis. Therefore, this study was undertaken in studies when the plantaris tendon is extirpated in the situation with Achilles tendinosis. The signal substance system on that was focused on was the TNF-alpha system. The reason is that TNF-alpha is known to be involved in blood vessel remodelling and angiogenesis (Baluk et al., 2009); (Ligresti et al., 2011). We have also previously observed that the tenocytes of the human Achilles tendon show expression of TNF-alpha as well as TNF receptors (Gaida et al., 2012).

Antibodies against TNF-alpha and TNF receptor II (TNFRII) were applied. The hypothesis was that the TNF-alpha system is involved in the processes in tendinosis, including in the blood vessel remodelling.

2 MATERIAL & METHODS

2.1 Individuals

Patients suffering from longterm pain (>3 months) in the Achilles tendon midportion were included. Examinations via ultrasound+Doppler showed thickening, irregular tendon structure but also hypoechoic regions and high blood flow localized outside and inside the ventral midportion part indicating Achilles tendinosis. The evaluated material consisted of samples from 7 patients: 6 men with a mean age of 40.2 years and 1 woman with an age of 58. The samples conformed to specimens of the plantaris tendon with attached peritendinous connective tissue. For control purposes a specimen from an individual without pain symptoms was evaluated as well (female, 27 years).

2.2 Sampling

During the surgery, the patients were kept under local anaesthesia (Pilokainhydrochloride 4-5 ml, 10 mg/ml, Astra Zeneca, Södertälje, Sweden). The procedure was as follows: Via a short longitudinal skin incision on the medial side the Achilles tendon was visualized. The plantaris tendon was in these cases discovered to lie very close to the Achilles tendon's medial and ventral part. In some cases it was even seen to be invaginated. Then the plantaris tendon was carefully freed distally and proximally and finally cut at both ends. The tendon tissue was accompanied by closely attached peritendinous tissue. The Achilles tendon was thereafter "scraped" in the regions with high blood flow on the ventral side according to currently outlined procedures (Alfredson, 2011c). For control purposes a plantaris tendon from healthy individual (female, 27 years)

was taken as well (c.f. above). Ultrasound+Doppler showed no pathological features in this case. Due to ethical reasons the obtaining of control tissue was restricted in this case.

The study protocol was approved by the Regional Ethical Board in Umeå (dnr 04-157M; 2011-83-32M). The experiments were conducted according to the principles expressed in the Declaration of Helsinki.

2.3 Fixation, Sectioning and Staining for Morphology

The procedures for fixation and sectioning are in accordance with previously described procedures for tendon specimens (Spang et al., 2013); (Gaida et al., 2012). For demonstration of morphology, sections were stained with haematoxylin and eosin (H&E).

2.4 Immunofluorescence Processing

Immunostainings for detecting immunoreactions for TNF-alpha and TNFRII were performed. The procedures conform to those previously used in our laboratory for the demonstration of these factors (Gaida et al., 2012). As secondary antiserum, fluorescein isothiocyanate (FITC)-conjugated AffiniPure donkey antigoat IgG (1:100) (code no: 705-095-003, Jackson Immune Research, West Grove, Pa., USA) was used. Examination was carried out in a Zeiss Axioscope 2 plus microscope equipped with epifluorescent technique and an Olympus DP70 digital camera. The antibodies used are goat polyclonal antibodies from Santa Cruz Technology (Santa Cruz, CA, USA). The antibody for detecting TNF-alpha (L-19, code no.: SC-1350) was used at a dilution of 1:100 in PBS. The antibody against TNFRII (C-20, code no.: SC-1074) is primarily targeted to the C-terminus of human TNFRII. It was diluted 1:50 in PBS. For further information on the antibodies, staining procedures and control stainings, see Gaida et al., (2012).

3 RESULTS

3.1 Morphology

Microscopical analysis of the Htx-Eosin treated sections showed that the samples contained tendon tissue and also parts of the peritendinous connective tissue. There was a large number of fine and large blood vessels in the peritendinous connective tissue (Figure 1). There were also very frequent cells in the tissue. The cells have in a previous study been found to be stained with antibodies against fibroblast marker or macrophage marker (Spang et al., 2013). There was also a large number of blood vessels within the tendon tissue Compared to the control specimen the loose connective tissue from tendinosis patients contained a much higher number of blood vessels and more cells.

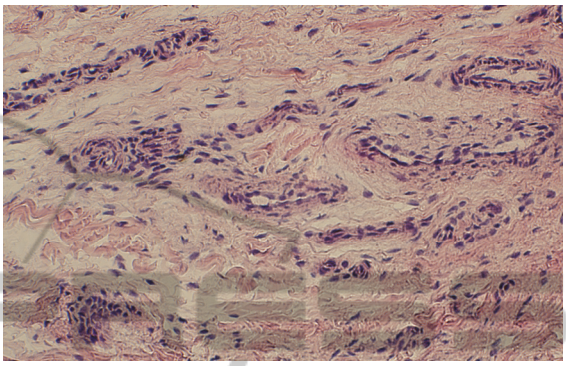

Figure 1: Peritendinous connective tissue between the plantaris and the Achilles tendon stained for haematoxylin and eosin. There is a marked presence of blood vessels.

3.2 Immunohistochemistry

3.2.1 TNF-alpha

Immunoreactions for TNF-alpha could be observed in cells in the peritendinous connective tissue (Figure 2a) and in tenocytes in the tendon proper (Figure 2b) in the tendinosis specimens. Weak reactions could be detected in the walls of blood vessels located in the tendon proper (Figure 2c) and in the peritendinous connective tissue (Figure 2d) in theses specimens. The immunoreactions in the cells in the peritendinous connective tissue were very finely point-like. Immunoreactions could also be observed in the occasionally seen cells in the peritendinous tissue, to some extent in blood vessel walls and in tenocytes in the control specimen (not illustrated).

3.2.3 TNFRII

TNFRII immunoreactions could be detected in the same type of structures as referred to above in the tendinosis specimens, namely the cells in the peritendinous connective tissue, the blood vessel walls and in tenocytes (Figure 3a-d). The reactions were strong and seen as small bright dots. Particularly, the blood vessel reactions were very marked.

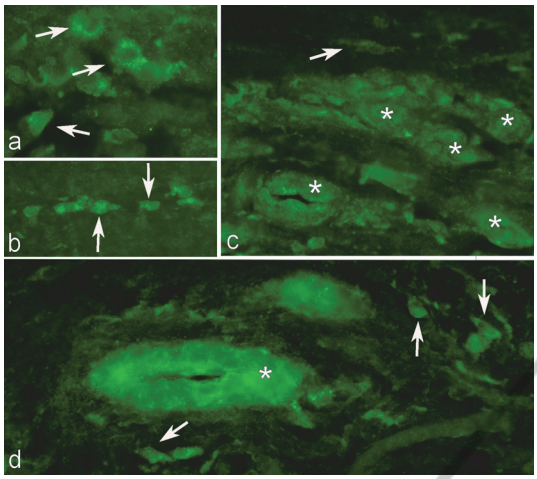

Figure 2: Immunolabelling for TNF-alpha. Plantaris tendon with attached peritendinous connective tissue is shown. Immunoreactions are observed in cells in the peritendinous connective tissue (a, arrows). Tenocytes in the tendon proper do also show specific reactions (b, arrows). Blood vessels in the tendon proper (c) and in the peritendinous connective tissue (d) are weakly positive for TNF-alpha (asterisks). Arrows at immunoreactive tenocyte in (c) and at immunoreactive cells in the peritendinous connective tissue in (d).

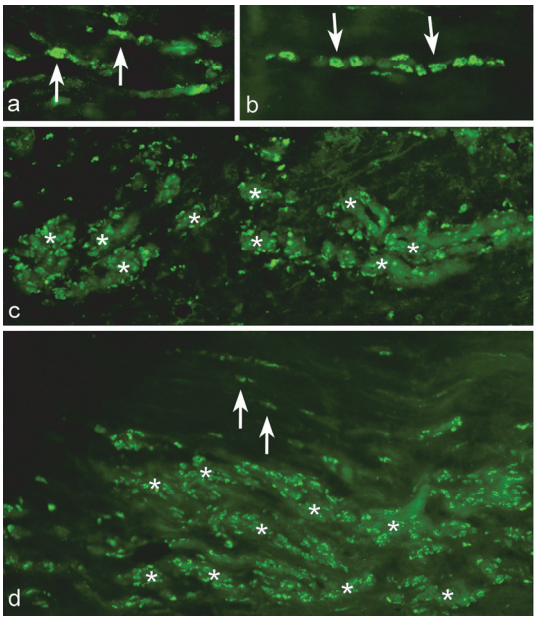

Figure 3: Immunolabelling for TNFRII. Plantaris tendon with attached peritendinous connective tissue is shown. Immunoreactions are seen in the cells in the peritendinous connective tissue (a, arrows) and in tenocytes in the tendon proper (b,d arrows). Furthermore very marked immunoreactions are seen in the walls of blood vessels in both the peritendinous connective tissue (c, asterisks) and the tendon proper (d, asterisks).

There were thus areas in the peritendinous connective tissue and in tendon tissue proper that exhibited widespread TNFRII reactions (Fig.3c,d). Immunoreactions were also noted for the blood vessel walls and to some extent for the tenocytes and the cells in the peritendinous connective tissue in the control specimen.

4 DISCUSSION

These evaluations show for the first time the occurence of TNF-alpha and TNFRII immuno-reactions in the plantaris tendon and in the peritendinous connective tissue between the plantaris tendon and the Achilles tendon in cases with Achilles tendinosis. It is evident that there is a local TNF-alpha production in cells in this tissue and that TNF-alpha is involved in the blood flow regulation of the tissue. There were thus very marked TNFRII reactions in the vessel walls. To some extent there were similar reaction patterns in the control specimen. However, it should be underscored that the vessels and the cells in the peritendinous connective tissue were much fewer than what was case for the tendinosis specimens. Due to ethical reasons, the obtaining of control samples was restricted to one individual.

Concerning the marked TNFRII immuno-reactions seen for blood vessel walls it is noteworthy that this TNF receptor is shown to enhance angiogenesis under low oxygen conditions (Luo et al., 2006). TNF-alpha is on the whole known to be involved in blood vessel regulation. It is e.g. shown that TNF-alpha is involved as an early component in the cascade leading to angiogenesis in response to aortic injury (Ligresi et al., 2011). The origin of the TNF-alpha in this case was macrophages (Ligresi et al., 2011). Renal ischemia is shown to be accompanied by increased expressions of TNF-alpha and TNF receptors, an increased expression of TNFRII being observable for the renal arteries and the neuroretina (Gesslein et al., 2010).

TNFRII, as well as TNF-alpha, immuno-reactions were also observed for tenocytes of the plantaris tendons. The situation is thus the same as was previously observed for the human Achilles tendon (Gaida et al., 2012). This can imply that autocrine/paracrine TNF-alpha effects occur for the tenocytes, effects which can be related to trophic functions. In accordance with this suggestion, it is known that binding at TNFRII is related to tissue repair, growth-modulating effects and differentiation (Ihnatko and Kubes, 2007). Due to the “compression

theory" the compressive forces on the peritendinous tissue can be very strong in Achilles tendinosis (Cook and Purdam, 2009). Therefore, tissue repair, growth-modulating influences and differentiation might play key roles during the tendinosis condition.

TNF-alpha blockers have been tested for patients suffering from chronic Achilles tendinopathy (Fredberg and Ostgaard, 2009). The clinical implication however is still unclear. The results of the present study indicate that further studies should be undertaken concerning the use of anti-TNF treatments.

In conclusion it is here shown that there is a marked presence of the TNF-alpha system in the situation with plantaris tendon involvement in Achilles tendinosis. TNF-alpha is produced in the peritendinous connective tissue and may be highly involved in the blood vessel remodelling as well as for tenocyte function. These findings stress that the TNF-alpha system might be an important system in a condition that is frequently involved for sports active persons, namely Achilles tendinopathy/ tendinosis.

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