Histol Histopathol (2009) 24: 1207-1211

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Tenascin-C and type I and III collagen expression in total Achilles tendon rupture. An immunohistochemical study

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Summary. Tendon tissue degeneration and changes in collagen composition play a role in spontaneous rupture of the human Achilles tendon. Tenascin-C has been shown to be present in the tissue pathology and changes in tissue loading. We made an immunohistological study of the expression of tenascin-C and type I and III collagens in ruptured human Achilles tendons. METHODS: Three tissue samples in ten individuals, one from the Achilles tendon rupture and two control samples from four and sixteen centimeters proximal in same tendon were collected at surgery. The specimen were fixed and labelled with specific antibodies to type I and III procollagens (PICP, PINP and PIIINP), mature type III collagen (IIINTP) and tenascin-C. The amount of reacting tissue was evaluated visually and graded on a semiquantitative scale. RESULTS: No difference in the expression of tenascin-C was found between the sites. Instead, mature type III collagen content (p=0.008) and type III collagen synthesis (p=0.016) were significantly increased at the rupture site relative to the control site 2. The amount of newly synthesized type I collagen (PINP, PICP) was relatively high at all sites, as expected. CONCLUSION: The expression of type III collagen is increased at the rupture site in the human Achilles tendon, but that of tenascin-C remains unchanged. This finding supports a tissue composition alteration background for Achilles tendon rupture, while the role of mechanical loading at the rupture site remains controversial.

Key words: Tenascin-C, type I collagen, Type III collagen, Achilles tendon, Rupture and degeneration

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Introduction

Although a healthy Achilles tendon can withstand loads up to 5000 Newtons (Gerdes et al., 1992), even a slight change in the tissue composition can predispose the tendon to rupture. Degenerative changes have been held responsible for total rupture of the human Achilles tendon, as supported by tendon histology studies, in which abnormal orientation of the collagen fibres, changes in collagen type composition and mucoid and hypoxic-type have been found (Kannus and Jozsa, 1991; Jozsa and Kannus, 1997).

The role of tenascin-C, one of the large extracellular matrix proteins in various tissues, has been a matter of great interest during the past 20 years. It is expressed in tissues during normal embryogenesis (Chiquet-Ehrismann et al., 2002) and several studies have discussed its presence in certain pathological conditions (Mackie et al., 1988; Chiquet-Ehrismann, 1993, 2002; Kaarteenaho-Wiik et al., 2002; Chiquet-Ehrismann and Chiquet, 2003). Its expression is practically non-existent in healthy connective tissue without mechanical loading, but it has been shown to be elevated after mechanical stimulus and in degenerative and reparative processes (Settles et al., 1996; Kannus et al., 1998; Jarvinen et al., 1999). Stressed fibroblasts have also been shown to express more tenascin-C in vitro (Ehrismann et al., 1994).

Type I collagen is the major substance in tendon tissue and is considered to be responsible for its mechanical strength, while type III collagen has a role in tendon tissue healing (Liu et al., 1995). Elevated amounts of type III collagen have been reported at the rupture site of the human Achilles tendon (Jozsa and Kannus, 1997; Eriksen et al., 2002). The presence of this collagen type is thought to cause weakening in the tendon tissue, since its fibres are thinner and more extensile than those of type I.

We set out here to test the hypothesis that there will be a high local expression of tenascin-C and type III collagen at the rupture site of the human Achilles tendon relative to two other sites in the same tendon.

Material and methods

Patient samples

Tissue samples were harvested during surgery for total Achilles tendon rupture. The patients were ten consecutive individuals all operated on using the one-flap augmentation technique (Silfverskiöld, 1941). The first sample was taken from the rupture site (RUPTURE), the second from lower end of the flap (CONTROL1) and the third from the uppermost corner of the flap (CONTROL2) (Fig. 1). The exclusion criteria were a previous Achilles tendon injury, age over 60 years, use of local or systemic corticosteroids and more than 48 hours elapsing between rupture and operation.

The protocol was approved by the Research Ethics Committee of Oulu University Hospital. All the patients received oral and written information and freely given informed consent was obtained from them in writing.

Tissue preparation

All the tissue samples were fixed in 10 percent buffered formalin, embedded in paraffin and cut to a thickness of 5 µm. Immunohistochemical staining was carried out by the avidin-biotin-immunoperoxidase technique, and the tissue sample slides were counterstained with haematoxylin-eosin. A monoclonal mouse antibody reacting to two major isoforms of human tenascin-C was employed to visualize tenascin.

Polyclonal antibodies were raised in rabbits, cross-absorbed with other connective tissue antigens and purified by immunoabsorption on the relevant antigens coupled to Sepharose 4B. These were used to characterize the type I and III procollagens and collagens

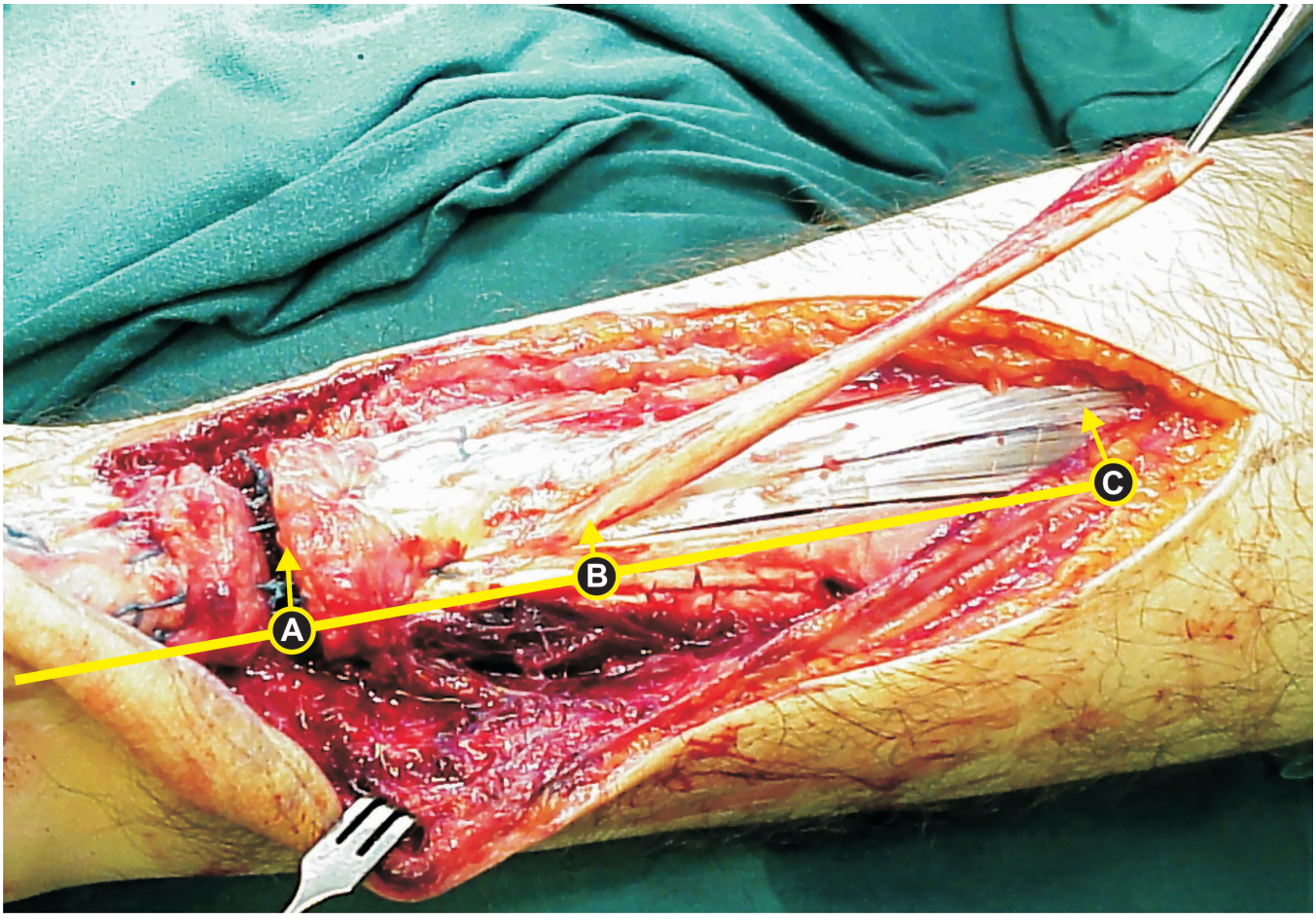

Fig. 1. Locations of tissue samples taken from Achilles tendon. A is the rupture site, B is control 1 and C is control 2. The distances from the heel bone insertion are A 4 cm, B 8 cm and C 16 cm. As seen in the picture, tendon-like tissue is still available at site C.

deposited in the tissue samples. Anti-PINP detects the aminoterminal propeptide of type I procollagen, and a positive staining indicates newly synthesized type I collagen still having the aminoterminal propeptide attached. This form is also called type I pN collagen. Anti-PICP detects the carboxyterminal propeptide and is also used as a marker of type I collagen synthesis. Anti-PIIINP detects the aminoterminal propeptide of type III collagen, which can be found in free form in the extracellular matrix or as type III pN collagen on the surfaces of type III collagen fibrils. It is used as marker of type III collagen synthesis. Anti-IIINTP detects the type III collagen, which is bound to collagen fibrils with normal intermolecular cross-linking.

The amounts of tenascin-C, type I and III procollagens and collagens were determined by analysing the immunoreactivity of the tissue sections, the evaluation being performed on a semiquantitative scale from 0 to 3, corresponding to the abundance of labelled tissue in the samples (0: reactivity absent; 1: under 33%; 2: 33-66%; 3: over 66%). The reactivity was evaluated independently by two pathologists (Fig. 2).

Statistical analysis

The statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, IL). The data are expressed as frequencies of rankings on the semiquantitative scale. Statistical significance in rankings between the three studied sites was calculated using Friedman's test. P-values according to Sign test for comparison of rupture and control sites were calculated

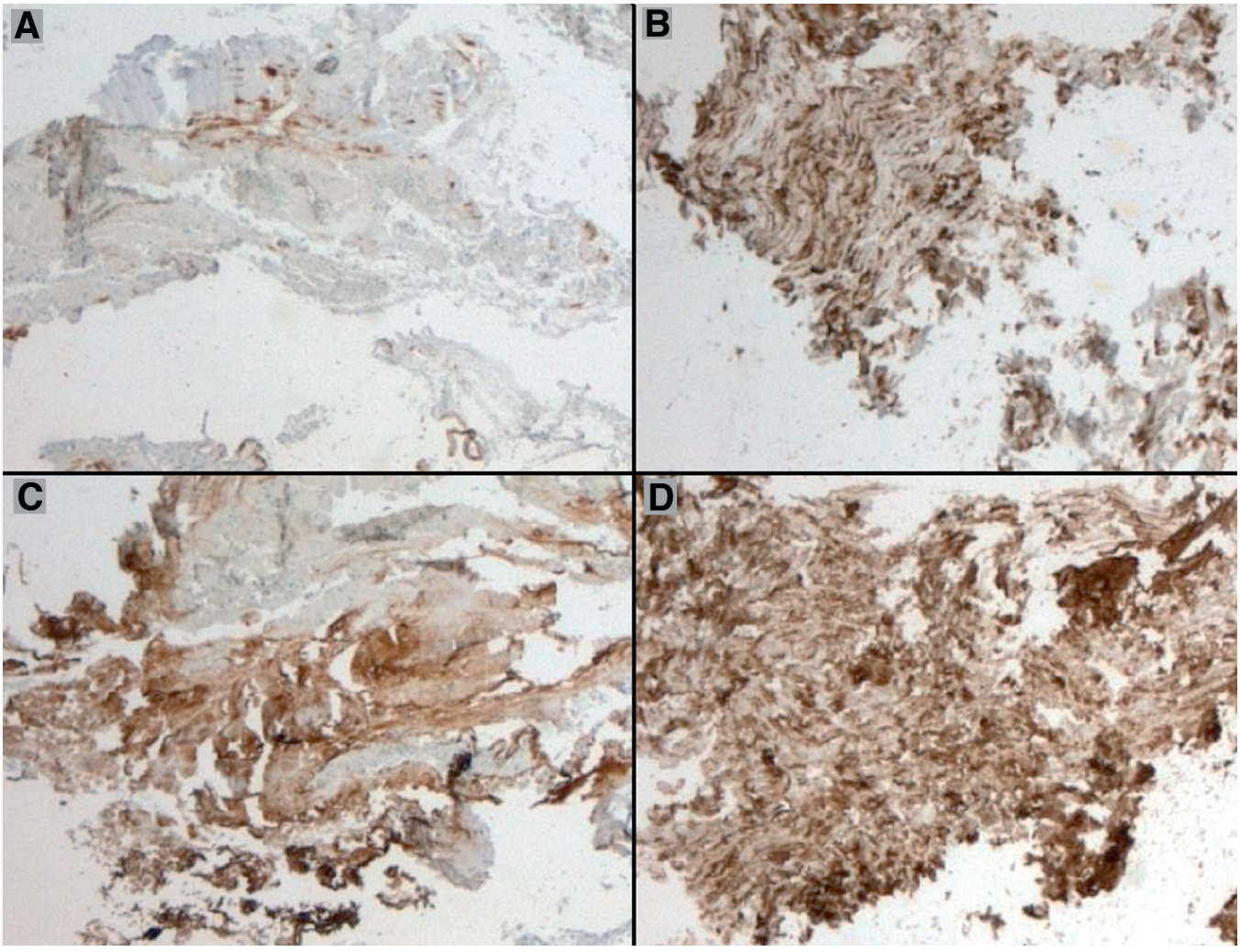

Fig. 2. Microscopy photographs of immunohistologically stained specimen show expression of tenascin-C level 1 (under 33% of surface area coverage) in Achilles tendon in picture **A** and tenascin-C level 2 (33 –66% of surface area coverage) in picture **B**. Expression of PINP in Achilles tendon level 2 in picture **C** and level 3 (over 66% of surface area coverage) in picture **D**. Optical magnification, x 4

Table 1. The expression of tenascin-C, type I and III procollagens and mature type III collagen at the studied sites. Figures represent frequencies in different scales used (0-3).

	Rupture site rankings				Control 1 rankings				Control 2 rankings				*p*
Frequency in different scales (0-3)	0	1	2	3	0	1	2	3	0	1	2	3	
PIIINP			5	4		6	1	3		7	3		0.021* 0.29‡ 0.016†
PINP		1	2	6			1	8				9	0.33*
PICP		3	4	2		3	3	4		3	3	4	0.60*
IIINTP		1	2	6		3	6	1		7	3		0.001* 0.070‡ 0.008†
Tenascin		7	1	1		9	1			6	4		0.40*

*Statistical significance in rankings between the three studied sites (Friedman test). ‡,† P-values according to Sign test for comparison of rupture and control 1 (‡) and 2 (†) sites (calculated if Friedman's test p<0.05).

when Friedman's test had p-value <0.05.

Results

Samples

The final series included nine men and one woman, with an average age of 38 years (range 30-48). They were operated on a mean of 29 (10-43) hours after the injury. One sample taken at the rupture site and one sample in the type I collagen analysis was spoiled during processing.

Tenascin-C

There was no significant difference between the sites (Table 1).

Type I and III procollagens

The type I carboxyterminal (PICP) and amino-terminal (PINP) procollagens were almost equal in expression at the sites (Table 1), but the expression of type III procollagen (PIIINP) was significantly higher at the rupture site than at control site 2 (Table 1, p=0.016 Sign Test).

Mature type III collagen

The amount of mature type III collagen present was significantly higher at the rupture site than at control sites 1 and 2 (Table 1, P=0.008 Sign Test).

Discussion

The main purpose here was to examine the patterns of tenascin-C and type I and III collagen expression in the ruptured human Achilles tendon by comparing expression at the rupture site with two other sites within the same tendon. The tendon samples were harvested less than 43 hours after rupture and should at most represent the tendon tissue composition before the trauma (Haukipuro et al., 1987; Fluck et al., 2000).

Although Riley et al. (1996) found elevated expression of tenascin-C in certain regions of degenerated human supraspinatus tendons, our results show no differences between the sites in this respect. Similarly, Jarvinen et al. have recently demonstrated higher tenascin-C expression at the musculo-tendinous junction of a rat Achilles tendon after increased physical loading (Kannus et al., 1998). We think that the long-term mechanical loading affecting the rupture site of the human Achilles tendon does not differ from that at other sites in the same tendon. Since any changes in tenascin-C expression should take place after a delay of more than 36 hours (Fluck et al., 2000), the levels obtained in the present ruptured tendons must mainly have originated from the time before the rupture. We suspect that there may be a more universal mechanical loading pathology associated with the degenerative process and rupture of the human Achilles tendon. Abnormal tension in the gastrocnemius apparatus could be one explanation for our findings, and we would agree that tenascin-C has some as yet unknown role in the loading changes taking place in connective tissue (Jarvinen et al., 2000).

Since we could not find any correlation between the expression of tenascin-C and type III collagen synthesis or accumulation, we believe that the role of tenascin-C in tendon degeneration is variable.

The levels of both mature and newly synthesized type III collagen were markedly higher at the rupture site than at the two control sites, as found in previous studies (Eriksen et al., 2002). The amount of type I collagen was the same at all three sites.

In conclusion, Type III collagen expression is elevated at the rupture site of the human Achilles tendon, whereas tenascin-C is expressed evenly over the whole tendon. We believe that tenascin-C has no specific value as a predictive or diagnostic marker of tendon degeneration. Our findings support results in which

tenascin-C expression has been shown to be elevated after mechanical loading, but the exact function of tenascin-C in the extracellular matrix of human tendon tissue still remains unsolved.

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Accepted March 27, 2009