

Linköping University Medical Dissertations, No. 1057

# Towards surgical use of matrix metalloproteinase biology

Björn Pasternak

Orthopaedics  
Department of Clinical and Experimental Medicine  
Linköping University  
Linköping, Sweden



Linköping University

Linköping 2008

©Björn Pasternak 2008

Cover picture by Anna Missios.

All previously published papers were reproduced with permission from the publishers.

Printed by LiU-Tryck, Linköping 2008.

ISBN: 978-91-7393-931-7

ISSN: 0345-0082

# CONTENTS

Abstract	5
List of papers	7
Introduction	9
Overview	9
Tendons	10
Matrix metalloproteinases	11
Tendinopathy	16
Tendon healing	19
Tendon suturing	19
Anastomotic leakage	20
Prediction of healing by MMPs	20
MMP-inhibitors	21
Aims, with background in brief	23
Methods	25
Results in brief	31
Discussion	37
Speculations and future research	47
Dåligt läkkött	49
Summary in Swedish	51
Acknowledgements	53
References	55
Papers I-IV	
Thesis at a glance	back cover



# ABSTRACT

Matrix metalloproteinases (MMPs), such as collagenases, are a family of enzymes capable of degrading most constituents of the extracellular matrix. MMPs are thought to be involved in the aetiopathogenesis of tendon rupture. Additionally, failure of healing has in some instances been associated with elevated levels of MMPs. We have studied (a) the effects of the MMP-inhibitor doxycycline on healing of tendons and intestines in experimental models and (b) systemic levels of MMPs and their endogenous inhibitors (TIMPs) in patients with tendon rupture.

In the first study, systemic doxycycline treatment lead to weakened rat Achilles tendons during healing after injury.

Subsequently, systemic doxycycline was shown to improve biomechanical properties of tendon suture fixation in the rat Achilles tendon. Sutures were also coated with doxycycline, leading to similar improvement in mechanical strength of the suture construct during healing.

In the third study, doxycycline-coated sutures improved the strength of healing intestinal anastomoses in an experimental model.

Finally, we showed that patients with a history of Achilles tendon rupture had elevated levels of MMP-2, MMP-7 and TIMP-2 in serum. In addition, MMP-7 correlated inversely to mechanical strength of the tendon during healing.

In conclusion, MMP-inhibitors can be administered systemically and locally to manipulate healing of tendons and intestines. Generalised alterations in the MMP-TIMP system may be involved in the pathogenesis of Achilles tendon rupture and associated with differences in outcome of healing.

**Key words:** Achilles tendon, colon, colorectal surgery, extracellular matrix, humans, matrix metalloproteinases (MMPs), rats, surgical anastomosis, sutures, tendon injuries, tetracyclines, tissue inhibitor of metalloproteinases (TIMP), wound healing.



# LIST OF PAPERS

This thesis is based upon the following papers, which will be referred to by their Roman numerals.

- I. Björn Pasternak\*, Mårten Fellenius\*, Per Aspenberg.  
**Doxycycline impairs tendon repair in rats.**  
Acta Orthop Belg 2006; 72: 756–60.
- II. Björn Pasternak, Anna Missios, Agneta Askendal, Pentti Tengvall, Per Aspenberg.  
**Doxycycline-coated sutures improve the suture-holding capacity of the rat Achilles tendon.**  
Acta Orthop 2007; 78: 680–6.
- III. Björn Pasternak, Martin Rehn, Line Andersen, Magnus S Ågren, Anne-Marie Heegaard, Pentti Tengvall, Per Aspenberg.  
**Doxycycline-coated sutures improve mechanical strength of intestinal anastomoses.**  
Int J Colorectal Dis 2008; 23: 271–6.
- IV. Björn Pasternak, Thorsten Schepull, Per Aspenberg.  
**Elevation of systemic matrix metalloproteinase-2 and -7 and tissue inhibitor of metalloproteinases-2 in patients with a history of Achilles tendon rupture.**  
Submitted

\*equal contribution



# INTRODUCTION

## Overview

---

Matrix metalloproteinases (MMPs) appear to be involved in the pathogenesis of several conditions involving the extracellular matrix. A better understanding of the roles of MMPs in tissue injury and the effects of their inhibition has the potential to improve outcome after injury and surgery. This thesis seeks to increase this understanding in order to provide the surgeon with novel tools.

Tendon rupture occurs in tendons that are altered by a degenerative process, in which their normal structure is broken down and replaced by disorganised connective tissue. This process is thought to be mediated in part by MMPs. One study in this thesis has dealt with the role of systemic MMPs in patients with tendon rupture. Since MMP enzymes are known to participate in all phases of tissue healing, we also addressed the hypothesis that variations in levels of MMPs are linked to variations in parameters of mechanical strength of the tendon during healing. In an experimental model, we have also studied whether inhibition of MMPs has any effect on mechanical characteristics of tendons during healing.

There are several mechanisms that contribute to the upregulation of MMPs in the clinical setting of acute tendon rupture. Firstly, induction occurs as an effect of the

tissue damage itself. Secondly, the insertion of the suture evokes a tissue reaction, and there is evidence of elevated MMPs at the tendon-suture interface. Thirdly, application of a plaster cast, i.e. unloading, leads to an increase in the expression of MMPs. Since these enzymes degrade the extracellular matrix, the weakening of tendons after injury, suturing and unloading should all principally be the result of increased MMP activity. Specifically, the upregulation of MMPs in the direct vicinity of the suture probably allows the suture to cut through the tendon when exposed to tensile stress. With this background, we have studied the effect of an MMP-inhibitor on suture fixation in tendons.

An intestinal anastomosis is constructed after resection of a segment of the large bowel. Interestingly, there are similarities between failure of the suture construct in tendons and failure of the anastomosis during healing (anastomotic leakage). MMPs have been established as important mediators of tissue breakdown in this common and serious complication after colorectal resection. One study in this thesis has addressed the effect of MMP-inhibition in an experimental model of colonic anastomosis healing.

## Tendon structure

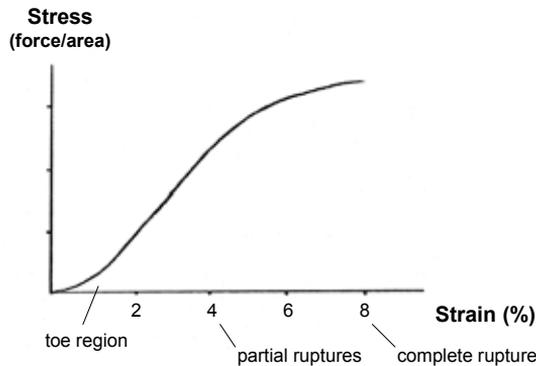
Tendons are composed out of collagens, proteoglycans and glycoproteins.

---

The smallest structural unit in tendons is the fibril, which largely consists of strictly organised collagen molecules. Tendon fibrils make up the fibres. Fibres in turn, are bundled to represent the fascicles, which are enclosed in the endotenon which supplies blood vessels and nerves to the tendon. Bundles of fascicles are surrounded by the epitenon, which is continuous with the endotendon. Tendon fibre bundles exhibit a crimp pattern. The stretching out of the crimp accounts for the toe region of tendon stress-strain curve (Figure 1), and thus protects against fibre damage. The ability for elastic deformation is limited. It is thought that the tendon withstands up to 4 % strain, thereafter partial ruptures start to develop in the fibrils.<sup>1</sup>

The basic constituents of tendon fibres are collagens, proteoglycans and glycoproteins.

Collagens provide the raw mechanical strength of the tendon. Collagen I is the dominating collagen subtype representing approximately 95 % of the total collagen, while collagen III is the second most common (~3 %). Proteoglycans, e.g. decorin, biglycan and aggrecan, carrying glucosaminoglycans as side chains, link together and demarcate the collagen fibrils. They also provide water-binding characteristics and resist compressive load. Tenascin C is another example of the functional importance of non-collagenous structural proteins. This glycoprotein is found throughout the tendon and is thought to play a role in the organisation and orientation of the extracellular matrix (ECM).<sup>2</sup> It appears to be important for elasticity and responds to mechanical load by increased synthesis, which is also regulated by growth factors and cytokines.<sup>2</sup>



**Figure 1.** Tendon stress-strain curve.

## Matrix metalloproteinases and their endogenous inhibitors

The extracellular matrix turnover is a dynamic equilibrium between synthesis and degradation. Degradation is principally mediated by MMP enzymes, which are antagonised by TIMPs.

---

Matrix metalloproteinases (MMPs) are a family of at least 24 zinc-dependent endopeptidases capable of degrading practically all components of the extracellular matrix (Table 1). MMPs contribute to many physiological processes through modification of the ECM.<sup>3</sup> Recent insights suggest that MMPs also have a broader spectrum of function including regulation of the inflammatory response, e.g. through effects on chemokine and cytokine signalling and by release of neopeptides from the ECM.<sup>4</sup>

While most MMPs are secreted into the extracellular space immediately after synthesis as proenzymes (pro-MMP), some may also be stored within cells (e.g. MMP-9 in neutrophil granules), and others are bound to cell surface membranes (e.g. MT1-MMP). The pro-MMPs are activated by proteolytic cleavage in the extracellular space, and MMP-3 seems to be a key player activating other MMPs in this manner.<sup>5, 6</sup> Baseline production of MMPs is low. Synthesis of MMPs is induced by a broad range of stimuli including cytokines (interleukin-1, -4, -6, -10, tumor necrosis factor- $\alpha$ ), growth factors, EMMPRIN\* and cell-cell or cell-matrix interactions, which all signal through intracellular pathways, such as the mitogen-activated protein (MAP) kinase pathway.<sup>7-11</sup>

The composition of the ECM depends on the balance between tissue formation and breakdown. The latter is mediated mostly by MMPs. Therefore, strict regulation of MMP production and activity is an essential part of ECM homeostasis. This regulation takes place at the levels of gene transcription, pro-

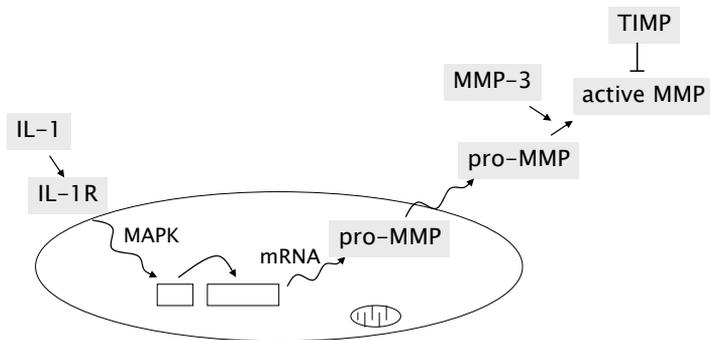
MMP activation and inhibition of active enzymes.

There are four tissue inhibitors of matrix metalloproteinases (TIMPs), which reversibly inhibit all MMPs by 1:1 interaction with the zinc-binding site.<sup>7</sup> TIMP-1, -2 and -4 are found in the tissues as well as in the circulation while TIMP-3 is sequestered in the ECM.<sup>12</sup> The specificity of TIMPs to individual MMPs is quite overlapping, although MT-MMPs appear to be resistant to TIMP-1.<sup>6, 12</sup> TIMPs have several functions besides MMP-inhibition, such as roles in regulation of angiogenesis and cellular proliferation.<sup>13</sup> Additional endogenous inhibitors of MMPs include the soluble proteins  $\alpha$ -1-antitrypsin and  $\alpha$ -2-macroglobulin,<sup>14</sup> as well as cell-membrane-linked MMP-inhibitors.<sup>12</sup>

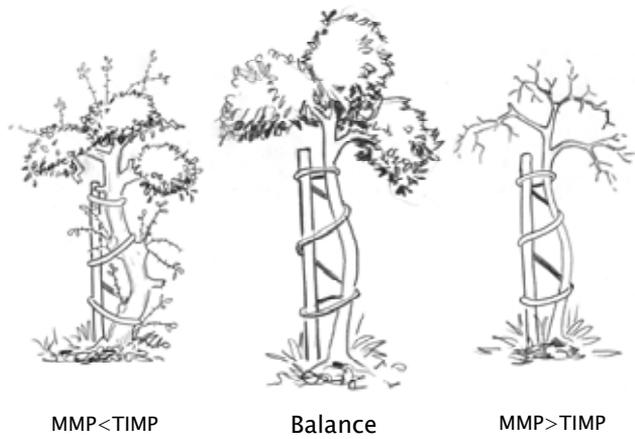
MMPs appear to have prominent roles in several diseases with a component of tissue destruction, such as osteoarthritis, rheumatoid arthritis and abdominal aortic aneurysm.<sup>15, 16</sup> It should be mentioned, however, that the view of MMPs as solely tissue degrading enzymes is somewhat oversimplified. This is exemplified by data from a case study on patients with a phenotype of multicentric osteolysis with e.g. carpal and tarsal resorption, arthritis and osteoporosis. These patients originated from consanguineous families, and were shown to have complete absence of pro- and active MMP-2 in serum, explained by two specific mutations in the MMP-2 gene.<sup>17</sup> This underlines the important physiological and developmental roles of MMPs.

---

\* EMMPRIN: extracellular MMP inducer



**Figure 2. Simplified drawing of the MMP system.** MMP gene transcription is typically induced by stimuli such as inflammatory cytokines, which signal via specific intracellular pathways. MMPs are produced as inactive pro-enzymes, which are subsequently cleaved to become active enzymes. MMP-3 appears particularly important in this regard, since it is known to activate several of the MMPs. TIMPs inhibit MMPs mainly at the active level. MAPK: mitogen activated protein kinase



**Figure 3.** Degradation of the extracellular matrix is principally mediated by MMPs, which are counterbalanced by TIMPs. Disturbances of this equilibrium may lead to disease processes of fibrotic (left) or degradative (right) nature.

**Table 1. The MMP family.** Compiled from refs<sup>4, 7, 14, 18-21</sup>. MMP-18 (collagenase 4) is not listed since it is considered a *Xenopus* collagenase, however, it has been detected in human ligaments.<sup>22</sup> Stromelysin 3 is grouped with “other MMPs”, since the enzyme has different properties from stromelysins. Among collagenous substrates for collagenases, bold numbers for collagens indicate strongest enzymatic activity. ADAMTS: a disintegrin and metalloproteinase with thrombospondin motifs, ECM: extracellular matrix, TGF: transforming growth factor, TNF: tumour necrosis factor, RASI: rheumatoid arthritis synovial inflammation, IGFBP: insulin growth factor binding protein, CXCL: CXC chemokine ligand.

Group	MMP	Collagenous substrates	Noncollagenous ECM substrates	Nonstructural ECM component substrates
<b>Collagenases</b>				
Collagenase 1	MMP-1	collagens I, II, III, VII, VIII, X, XI, gelatins	proteoglycans, fibronectin, entactin, laminin, tenascin, vitronectin	$\alpha$ -1-antiprotease, pro-TNF $\alpha$
Collagenase 2	MMP-8	collagens I, II, III, V, VII, VIII, X	fibronectin, laminin, proteoglycans	ADAMTS-1, pro-MMP-8
Collagenase 3	MMP-13	collagens I, II, III, IV, V, VII, IX, X, gelatins	proteoglycans, fibronectin, laminin, tenascin	fibrinogen, proMMP-9 and -13
<b>Gelatinases</b>				
Gelatinase A	MMP-2	gelatins, collagens I, II, III, IV, VII, X	laminin, elastin, fibronectin, proteoglycans	pro-MMPs -9 and -13, $\alpha$ -1-antiprotease, IGFBPs, IL-1 $\beta$ , TGF $\beta$
Gelatinase B	MMP-9	gelatins, collagens IV, V, VII, X, XI	laminin, elastin, fibronectin, proteoglycans	$\alpha$ -1-antiprotease, CXCL5, IL-1 $\beta$ , TGF $\beta$ , plasminogen
<b>Stromelysins</b>				
Stromelysin 1	MMP-3	collagens III, IV, V, VII, IX, X, XI, gelatins	laminin, fibronectin, elastin, proteoglycans	pro-MMPs, pro-TNF $\alpha$ , E-cadherin, L-selectin, fibrinogen
Stromelysin 2	MMP-10	collagens I, III, IV, V, IX, X, gelatins	laminins, proteoglycans	pro-MMPs
<b>Matrilysins</b>				
Matrilysin 1	MMP-7	gelatins, collagens I and IV	laminin, elastin, fibronectin, proteoglycans, tenascin	pro-MMPs, pro- $\alpha$ -defensin, pro-TNF $\alpha$ , E-cadherin
Matrilysin 2	MMP-26	as above	as above	as above
<b>Membrane-type (MT) MMPs</b>				
MT1-MMP	MMP-14	gelatin, collagens I, II, III	proteoglycans, fibronectin, tenascin, fibrinogen	Pro-MMP-2 and -13
MT2-MMP	MMP-15			Pro-MMP-2
MT3-MMP	MMP-16	gelatins, collagen III	fibronectin	Pro-MMP-2
MT4-MMP	MMP-17			
MT5-MMP	MMP-24		fibronectin	Pro-MMP-2
MT6-MMP	MMP-25	gelatin		
<b>Other MMPs</b>				
Stromelysin 3	MMP-11		fibronectin	$\alpha$ -1-antiprotease, serpins
Metalloelastase	MMP-12	collagens, gelatins	elastin, proteoglycans	plasminogen
RASI	MMP-19		components of basement membranes	
Enamelysin	MMP-20		amelogenin	
-	MMP-21	gelatin		
-	MMP-23			
-	MMP-27			
Epilysin	MMP-28			Pro-TGF $\beta$

## Tendinopathy and tendon rupture

---

### Tendinopathy

Tissue breakdown and regeneration are upregulated simultaneously.

Painful tendinopathy is common in patients consulting primary care and orthopaedic surgeons. Patients present with pain originating from e.g. Achilles, patellar or supraspinatus tendons. These conditions are thought to result from repetitive micro-trauma,<sup>23</sup> and are often described as overuse injuries. The typical histopathologic finding in patients presenting for surgery for painful Achilles tendinopathy is degeneration, i.e. disorganised tissue, variation in the density of tendon cells, ranging from hypercellularity to hypocellularity, and increase in vascularity.<sup>1, 24, 25</sup> The findings are consistently defined as non-inflammatory, which forms the basis for the histological description termed tendinosis, as opposed to tendinitis. Although there is little doubt that tendinosis is non-inflammatory, it has to be remembered that histological studies are invariably performed on tendons from patients who have come to the attention of surgeons, i.e. from patients with long standing painful tendinopathy, often longer than a year.<sup>26</sup> Thus, the biochemical processes that lead to the observed state are quite unknown and the inciting event could still have an inflammatory component.<sup>24</sup> In fact, in cellular models, tenocytes release proinflammatory cytokines and prostaglandins in response to repetitive mechanical overloading.<sup>23, 27</sup>

### DEFINITIONS<sup>28-30</sup>

#### Painful tendinopathy

A clinical condition caused by degenerative changes in the tendon extracellular matrix. If this condition causes trouble for a considerable amount of time, it is called chronic painful tendinopathy. Partial tendon tears also fall under the spectrum of painful tendinopathy. Tendinosis is a term to describe tendinopathy histopathologically. The terms tendinitis and tendonitis are no longer in use.

#### Tendon rupture

A complete rupture of the tendon

Maintenance of the biochemical composition of the ECM is essential for optimal structure and function of the tendon. Studies of tendinopathy show aberrant ECM composition and high ECM turnover rate, as evidenced by elevated MMP activity, markers of collagen turnover and collagen gene expression.<sup>26, 28</sup> Thus, tissue breakdown and regeneration are upregulated simultaneously.

Gene expression studies on biopsy samples have shown elevated levels of both collagen type I and III in painful tendinopathy.<sup>26</sup> Studies on tendon cell cultures from patients with painful tendinopathy and tendon rupture showed elevated immunostaining for type III collagen and decreased staining for type I collagen in both conditions, as compared to non-tendinopathic tendons.<sup>31</sup> An extensive gene expression mapping of tendon samples from patients with chronic painful Achilles tendinopathy showed elevated expression of MMP-11, -16 and -23, and downregulation of MMP-3, -10, -12, -27 and TIMP-3.<sup>32</sup> The finding of MMP-3 downregulation is especially interesting since this protease is considered an

important regulator of MMP activation. Its down-regulation might represent an attempt to limit total MMP activity as a response to excessive tissue damage. In another gene expression study, tendinopathic tendons had higher levels of proteoglycans.<sup>33</sup> This, together with reduced activity in one of the MMPs that degrades proteoglycans (MMP-3) and an altered regulation profile of the proteoglycanolytic ADAMTS<sup>†</sup>, may lead to net accumulation of proteoglycans. Since an increase in proteoglycans would lead to altered mechanical properties, this process might be involved in a vicious circle stimulating further tendinopathic changes. Tenascin C, a glycoprotein important for structural and biomechanical properties of the ECM, is also elevated in tendinopathy,<sup>34</sup> further supporting the view of the ECM in dysbalance.

Presence of neovascularisation is thought to signal chronic disease and forms the basis for the concept of sclerotherapy with polidocanol, which seems a promising method.<sup>35</sup> There are, however, conflicting results as to whether there is any correlation between pain level and neovascularisation.<sup>36, 37</sup>

Pain in tendinopathy might be mediated by the neurotransmitters glutamate and substance P (SP).<sup>38, 39</sup> Interestingly, SP upregulates the gene expression of MMPs and TIMPs in fibroblasts,<sup>40</sup> which may connect SP to the altered regulation profile of MMPs and TIMPs observed in tendinopathy. SP is also involved in repair processes and, when administered exogenously, appears to enhance proliferation of fibroblasts and tendon healing.<sup>41-43</sup>

Nitric oxide (NO) is very promising in the treatment of tendinopathy. Animal studies have shown that NO participates in healing and that inhibition of NO reduces mechanical strength of tendons during

healing, while the addition of NO improves healing. Three randomised trials have shown that NO, administered via a dermal patch, reduces symptoms in Achilles and supraspinatus tendinopathies, and in tennis elbow, with improvement most apparent in the long term.<sup>44</sup> The role of NO in the pathogenesis of tendinopathy is however unclear.

A couple of studies suggest that tendon disease is associated with variations in primary connective tissue composition; patients with painful Achilles tendinopathy differ from control subjects in a variant of the collagen Va gene,<sup>45</sup> and Achilles tendon rupture and painful tendinopathy are coupled to a single nucleotide polymorphism in the tenascin C gene.<sup>46</sup> In addition, patients who have suffered Achilles tendon rupture are at excessive risk of a new rupture in the contralateral tendon.<sup>47</sup> Thus, a genetic predisposition to Achilles tendon disease seems likely. This is supported by studies showing the importance of hereditary factors in rotator cuff rupture and anterior cruciate ligament injury.<sup>48, 49</sup> Tendinotic histological alterations have been shown not only in macroscopically tendinopathic Achilles tendon lesions in patients with tendinopathy, but also in apparently healthy portions of the Achilles tendons in these patients.<sup>26</sup> This further supports the view that tendon disease might be part of generalised alterations of the ECM.

### **Tendon rupture**

Tendon degeneration might lead to rupture

The annual incidence of Achilles tendon rupture is approximately 5 to 30/100000, with an incidence peak at around 40 years of age, affecting men more commonly than women.<sup>50-53</sup> Sudden high-load stress, e.g. a rapid turn during sport activity, is the typical direct cause of rupture. Degenerative changes are invariably found in ruptured tendons<sup>54</sup> and degeneration in ruptured tendons appears more severe than in

---

<sup>†</sup> ADAMTS: a disintegrin and metalloproteinase with thrombospondin motifs. These enzymes degrade e.g. proteoglycans.

tendinopathy.<sup>55</sup> Although there are many similarities between the histopathological appearances of tendons from patients with painful tendinopathy and tendon rupture, there are some biochemical and molecular findings that point towards the notion that these are two distinct entities. For example, the gene expression profiles of MMPs, TIMPs, ADAMs, ADAMTS and proteoglycans in ruptured tendons are somewhat different from the ones in painful tendinopathy.<sup>32, 33</sup>

The aetiology of tendon rupture is incompletely understood but several risk factors are recognised (Table 2). Treatment is either conservative, i.e. immobilisation in plaster cast for six to eight weeks, or surgical, i.e. suturing of the ruptured tendon ends followed by a similar period of immobilisation. There is still some controversy as to which treatment modality should

be first-line. A meta-analysis has shown that Achilles tendon re-rupture is more common in patients treated conservatively, while the incidence of complications such as infections and adhesions is increased in those treated surgically.<sup>56</sup> There is now also growing interest in the role of early (postoperative) motion and loading.<sup>57</sup>

Some risk factors for re-rupture have been proposed (Table 2), but a large proportion of patients who suffer re-rupture of the Achilles tendon do not have any of the suggested risk factors.<sup>58</sup> In large, the clinical outcome of tendon healing is inherently difficult to predict.<sup>59</sup>

Thus, there is some evidence for an aetiopathologic role of MMPs in tendon rupture and some reasons to suspect a genetic predisposition to tendon rupture.<sup>60</sup>

Rupture	Re-rupture
<ul style="list-style-type: none"> <li>• previous tendon rupture<sup>47</sup></li> <li>• polymorphism in tenascin C gene<sup>46</sup></li> <li>• male sex<sup>61</sup></li> <li>• sports activity, e.g. badminton<sup>52, 62</sup></li> <li>• corticosteroid use<sup>61</sup></li> <li>• fluoroquinolone antibiotic use<sup>61, 63 *</sup></li> <li>• osteoarthritis<sup>61</sup></li> <li>• autoimmune arthritis<sup>61</sup></li> <li>• gout<sup>61</sup></li> <li>• transplants/dialysis<sup>61</sup></li> <li>• blood group? <sup>64, 65</sup></li> </ul>	<ul style="list-style-type: none"> <li>• corticosteroid therapy?<sup>58</sup></li> <li>• age?<sup>58</sup></li> <li>• smoking?<sup>58</sup></li> <li>• delay in treatment?<sup>58</sup></li> </ul>

**Table 2. Suggested risk factors for tendon rupture and re-rupture.** Question marks signify preliminary or conflicting results. Factors are not listed in order of importance. \*Interestingly, fluoroquinolones have been shown to affect expression and activity of MMPs in tendon cells and in epithelial cells.<sup>66-68</sup>

## Tendon healing

A classical tissue healing process

---

Tendon healing occurs in three overlapping phases. Platelets form a clot and initiate the healing response. In the initial inflammatory phase neutrophils enter the site of injury. Macrophages arrive and clean up necrotic material. Vasoactive and chemotactic factors are released with increased vascular permeability, initiation of angiogenesis, stimulation of tenocyte proliferation, and recruitment of more inflammatory cells. Cells from the paratenon gradually migrate to the wound and after a few days, the proliferative phase begins. Synthesis of type III collagen peaks, and water content and glycosaminoglycan concentrations remain high during this stage. This is followed by the remodelling phase. Initially, the repair tissue changes from cellular to fibrous while tenocyte metabolism remains high, and

tenocytes and collagen fibres become aligned in the direction of stress. A higher proportion of type I collagen is synthesised during this stage. Later on, the fibrous tissue gradually changes from scar to a tendon-like tissue.

Only a few studies have dealt with the role of MMPs in tendon healing. Based on mRNA data from unloaded healing rat flexor tendons, it has been suggested that MMP-9 and -13 participate only in tissue degradation during the early phase of healing, while MMP-2, -3 and -14 participate both in tissue degradation and later remodelling.<sup>69</sup> This is largely supported by mRNA data from cutaneous wound healing.<sup>70</sup>

## Tendon suturing

The strength of the suture construct in tendons decreases transiently in the immediate postoperative period. A degradative process, mediated by MMPs, is thought to occur in the direct vicinity of the sutures.

---

In surgical repair, the ends of the ruptured tendon are sutured. The suture holding capacity of the repair site decreases in the immediate postoperative period by somewhere between 10 and 70 % (variation between mobilised and immobilised tendons and animal models).<sup>71-73</sup> The tendon tissue around the suture seems to be weakened,<sup>74</sup> which facilitates the suture to cut through the tendon when exposed to tensile stress, i.e. during mobilisation. Studies concerning finger flexor and Achilles tendon repair report a re-rupture rate of 3 to 6 %.<sup>75, 76</sup> Repair-site elongation and gap formation are more common, resulting in compromised healing and with that, poorer functional outcome in flexor tendons.<sup>77-79</sup> One study

also reports inverse association between tendon elongation and clinical outcome in Achilles tendon healing.<sup>57</sup> Improvement of suture techniques has decreased the initial weakening of the repair site, but it still is a problem in orthopaedic and hand surgery.

Implantation of a foreign material into the tendon invariably evokes a tissue reaction, and there is evidence of elevated MMP levels at the tendon-suture interface.<sup>74</sup> These enzymes degrade the extracellular matrix, which probably allows the suture to cut through the tendon. Inhibition of MMPs could thus serve to improve tendon suture holding capacity.

## Anastomotic leakage

In this life threatening complication after intestinal surgery, tissue breakdown is mediated largely by MMPs.

---

During surgery for pathologic alterations a piece of the intestine is removed and the two remaining ends are sewed or stapled together - an anastomosis is constructed. Although colorectal anastomoses usually heal well, in approximately 5-15 % of patients the intestinal ends do not hold together and intestinal contents spill into the abdominal cavity.<sup>80</sup> Anastomotic leakage remains a major unresolved problem in patients undergoing colonic or rectal resection. The strength of a newly constructed anastomosis is approximately 30 % of that of intact colon.<sup>81</sup> Pathophysiologically, the integrity of a newly constructed anastomosis is mainly determined by the suture holding capacity of the wound margins. This may deteriorate by as much as 50 % during the first few days after surgery, mainly because of degradation of extracellular matrix proteins through the action of MMPs.<sup>82, 83</sup> Several MMPs are strongly upregulated in the direct vicinity of the anastomotic suture line.<sup>84, 85</sup> Anastomotic MMP activity is yet higher in concurrent bacterial peritonitis, generating

further deterioration of anastomotic strength.<sup>83</sup> Furthermore, a recent study showed that patients with higher preoperative levels of MMP-1, MMP-2 and MMP-9 in the large bowel wall had an increased rate of anastomotic leakage.<sup>86</sup> This demonstrates the critical roles of MMPs as mediators of a decreased suture-holding capacity and indicates that MMPs are potential drug targets to improve anastomotic integrity. Accordingly, animal studies consistently show that the strength of the healing colon is enhanced by systemic MMP-inhibitors,<sup>82, 87, 88</sup> an effect most evident on the third postoperative day. There is only one published clinical study evaluating the effect of a protease inhibitor.<sup>89</sup> This randomised controlled trial failed to show any effect of the general protease inhibitor aprotinin on colorectal anastomotic leakage (see comments in discussion).

In summary, MMP-inhibitors appear to be promising pharmacological tools to prevent anastomotic leakage after colorectal surgery.

## Prediction of tissue healing quality by MMPs

---

Several studies have shown associations between alterations in MMP levels and poorer outcome of tissue healing. Preoperative MMP-9 in nasal secretions was shown to correlate inversely to healing quality after sinus surgery.<sup>90</sup> MMP-9 in wound fluid obtained at 24 hours after inguinal hernia surgery correlated inversely to collagen deposition at 10 days.<sup>91</sup> MMP-1, -2 and -9 in intestinal biopsies were higher in patients who developed anastomotic wound failure after colorectal resection.<sup>86</sup>

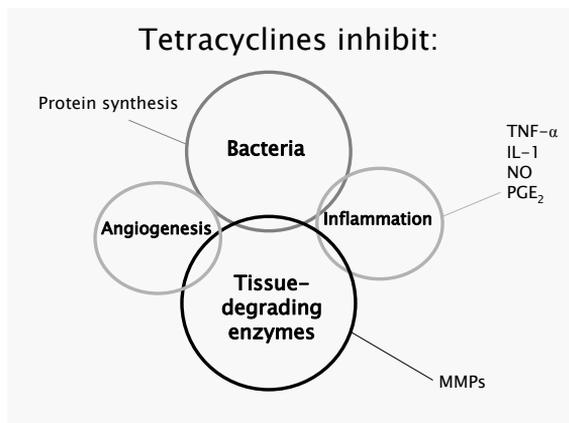
Serum MMP-1 and -8 were higher and TIMP-1 was lower in patients who developed non-union in long bone fracture healing.<sup>92</sup> A polymorphism in the MMP-1 gene was overrepresented in patients who developed aseptic loosening of hip prostheses.<sup>93</sup> These results not only show that MMPs are important in the pathogenesis of impaired healing by also indicate that MMPs are potential markers to predict outcome of tissue healing.

## MMP-inhibitors

Doxycycline is the most potent among the clinically available MMP-inhibitors

There are several classes of pharmacological MMP-inhibitors. The most common mechanism of action is binding to the zinc site of the MMP enzyme, thereby blocking its activity. The **tetracycline antibiotics** have several important non-antibiotic mechanisms (Figure 4).<sup>94-100</sup> Doxycycline is considered the most potent MMP-inhibitor among the tetracyclines and exhibits a broad spectrum by inhibiting MMPs -1, -2, -7, -8, -9, -12 and -13.<sup>99, 101</sup> Besides zinc-binding, tetracyclines also inhibit MMPs at the gene expression level and by reducing activation via the inflammatory cascade and via reactive oxygen species. **Chemically modified tetracyclines (CMTs)** have been developed to avoid unnecessary effects on the endogenous microbial flora while retaining the MMP-inhibitory action. Effects

on the microbial flora can also be avoided by administering “low-dose” doxycycline, i.e. 20 mg twice a day instead of the standard dose 100 mg twice a day. This way, doxycycline still retains its clinical (MMP-inhibitory) efficacy<sup>102, 103</sup> but has no effect on the vaginal or gut microbial floras.<sup>104</sup> **Synthetic MMP-inhibitors** constitute a large heterogeneous group of compounds, modified to increase inhibitory potency and to produce increased specificity against particular MMPs.<sup>105</sup> The bone resorption-inhibitors **bisphosphonates** also have potent MMP-inhibitory properties, probably working through cation-chelation.<sup>7, 106, 107</sup> Examples of MMP-inhibitors and experimental and clinical applications are presented in Table 3.



**Figure 4. Mechanisms of action of tetracyclines.** Tetracyclines have several non-antimicrobial effects. The ability of these drugs to inhibit matrix metalloproteinases is well established. Based on this mechanism, tetracyclines have come into clinical use for rheumatoid arthritis and periodontitis. Doxycycline and minocycline are the two tetracyclines that have been studied most extensively. TNF: tumour necrosis factor, IL: interleukin, NO: nitric oxide, PG: prostaglandin.

<b>MMP-inhibitor</b>		<b>Experimental studies</b>	<b>Clinical studies</b>
<b>Tetracyclines</b>	Doxycycline	Prevents weakening of mechanically unloaded tendons <sup>108</sup>	Effective as adjunctive therapy against rheumatoid arthritis (RCT). Same effect of low dose* (20 mg x 2) as standard dose (100 mg x 2) <sup>102</sup>
		Improves mechanical strength of intestinal anastomoses <sup>88</sup>	Low-dose* doxycycline approved as adjunctive therapy for periodontitis (several RCTs) <sup>103</sup>
		Antinociceptive and anti-inflammatory effects <sup>109</sup>	Slows the expansion rate of abdominal aortic aneurysms (RCT) <sup>113</sup>
	Minocycline	Effective in model of multiple sclerosis <sup>110</sup>	Improves symptoms in rheumatoid arthritis (RCT) <sup>114</sup>
		Reduces infarct size in stroke model <sup>111</sup>	Effective in pilot study for multiple sclerosis <sup>115</sup>
		Reduces hyperthrophic scarring <sup>112</sup>	
<b>Chemically Modified Tetracyclines (CMTs)</b>	CMT-3/COL-3	Prevents ARDS and shock in sepsis model <sup>116</sup>	Effective in phase II-trial for Kaposi's sarcoma <sup>117</sup>
<b>Synthetic MMP-inhibitors</b>	GM 6001	Improves mechanical strength of intestinal anastomoses and cutaneous wounds <sup>82, 118</sup>	Numerous synthetic MMP-inhibitors have failed in cancer trials due to either lack of efficacy or side effects <sup>101</sup>
	BB-1101	Improves mechanical strength of intestinal anastomoses <sup>87</sup>	
		Preserves brain anatomy and function from damage due to meningitis <sup>119</sup>	

**Table 3. Three major classes of pharmacological matrix metalloproteinase-inhibitors and examples of studies on the efficacy of these drugs in various experimental and clinical applications.**

\*Low-dose doxycycline (20 mg x 2) does not affect the composition or pattern of resistance of faecal and vaginal microbial floras in humans<sup>104</sup>. RCT: randomised controlled trial. ARDS: acute respiratory distress syndrome.

# AIMS, with background in brief

Background	Aim
MMPs participate in tendon healing. Drugs that inhibit MMPs are needed in tendon applications. The effect of an MMP-inhibitor on mechanical properties of the tendon during healing is not known.	To investigate the effect of the MMP-inhibitor doxycycline on experimental tendon healing.
Tendon suture-holding capacity decreases during the postoperative period. MMPs are thought to be involved.	To develop a rat model for the study of tendon suture-holding capacity and to investigate the effect of the MMP-inhibitor doxycycline on tendon suture fixation in this model, administered systemically and locally.
Strength of intestinal anastomoses decreases in the early postoperative period. Degradation is mediated by MMPs. Systemic MMP-inhibitors have positive effects on mechanical strength.	To investigate the effect of local administration of the MMP-inhibitor doxycycline on experimental colonic anastomosis healing.
MMPs are involved in the pathogenesis of tendinopathy and tendon rupture. Studies have suggested that there are possible systemic factors in the aetiology of tendon rupture.	To compare serum levels of MMPs and TIMPs between patients who have suffered Achilles tendon rupture and controls.
Healing of tendons varies between patients. Poor tissue healing has been associated with elevated levels of MMPs in some settings.	To investigate the association between baseline serum MMP and TIMP levels and biomechanical parameters of the tendon during healing.



# METHODS

Below is presented a condensed overview. Please refer to the papers for further details.

---

## STUDY DESIGNS

### Study I: Effect of doxycycline on rat tendon healing

Sixty female Sprague Dawley (SD) rats were randomised to receive doxycycline hyclate (130 mg/kg; Sigma Aldrich, St.Louis, USA) in the drinking water or no treatment. The left Achilles tendon was cut in all animals. Biomechanical evaluation was performed at 5, 8 and 14 days (n=10 per group) after surgery. Data were evaluated using two-way ANOVA, with Bonferroni post-hoc tests<sup>‡</sup>. Serum doxycycline concentration was determined in five randomly selected rats in the day 14 doxycycline-group.

### Study II: Effect of doxycycline on rat tendon suture fixation

All rats were subject to Achilles tendon surgery. The left Achilles tendon was transected, and a suture was inserted only into the distal portion of the cut tendon. Biomechanical suture pull-out strength was measured.

#### Systemic doxycycline treatment

In a pilot study, 20 male SD rats were randomised to receive doxycycline in the drinking water or no treatment. Biomechanical parameters were evaluated at 3 days postoperatively. Thereafter, 60 male SD rats were randomised to systemic doxycycline or no treatment and evaluated at 3, 5 and 7 days after operation. Data from the 80 animals together were evaluated by two-way ANOVA, without post-hoc tests. As a reference, the contralateral tendons of the day 3 control group were sutured

immediately after killing to serve as freshly inserted (day 0) controls.

#### Doxycycline-coated sutures

This substudy consisted of two experiments. In the first (Experiment 1), male SD rats were randomised to doxycycline-coated sutures (n=17) or uncoated control sutures (n=16). Biomechanical parameters were evaluated at three days postoperatively. As a reference, another 10 rats were operated and immediately evaluated to serve as freshly inserted (day 0) controls. In Experiment 2, male SD rats were randomised to doxycycline-coated sutures (n=24) or carrier (fibrinogen)-coated controls (n=24) and evaluated mechanically at 3 days postoperatively. Another 10 rats served as freshly inserted (day 0) controls. Data were analysed by two-way ANOVA, without post-hoc tests, with treatment as one independent factor and the two experiments as the other. Day 0 controls were not included in the statistical analyses.

### Study III: Effect of doxycycline-coated sutures on mechanical strength during healing of colonic anastomoses.

This study consisted of two experiments. In the first (experiment A), 40 male SD rats were randomised to three groups. A colonic anastomosis was constructed in all animals. Biomechanical properties of the colonic anastomoses treated with doxycycline-coated sutures (n=15) and carrier (fibrinogen)-coated sutures (n=15) were compared at three days postoperatively using Student's t-test. Additionally, biomechanical properties were evaluated directly after the operation of anastomoses constructed using carrier-coated sutures in 10 rats. These immediate day 0 controls were compared to day 3 controls by the Student's t-test to evaluate the decrease in

---

<sup>‡</sup> Post-hoc tests were not part of the evaluation in the original report, but added during preparation of this text.

anastomotic strength. As a reference, five additional rats were not operated on and used to determine the mechanical properties of uninjured colon.

In experiment B, 40 rats were randomised to equally sized groups which received uncoated sutures or carrier-coated sutures. Biomechanical properties were determined at three days postoperatively.

#### Study IV: Serum MMPs and TIMPs in patients with a history of Achilles tendon rupture

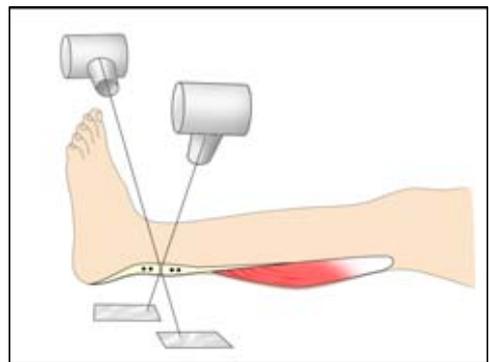
Eight patients who had participated in a prospective study concerned with biomechanical evaluation of Achilles tendon healing were recruited.<sup>120</sup> All patients were treated surgically in local anaesthesia, using the Kessler suture technique and fibrin glue to adapt the ruptured tendon ends. The original study included 10 consecutive patients but only eight of these were willing to participate in the current study. There were two women and six men, age range 35-52 years. Patients had suffered their Achilles tendon rupture a median 37 (range 35-40) months prior to the collection of blood samples for MMP and TIMP analyses. Exclusion criteria for providing blood samples were surgery or bacterial infection within the preceding month. Control serum was obtained from 12 healthy blood donors,

two women and ten men, age range 35-50 years. These subjects were recruited during routine blood donation. Their history concerning tendon disease was not known. MMP-1, -2, -3, -7, -8, -9 and -13, and TIMP-1 and -2 were determined in all subjects. Differences between the two groups were evaluated by Mann-Whitney U tests.

Small tantalum beads were implanted into the ruptured tendon ends at surgery and used as markers during biomechanical measurements (figure 5). Tendon mechanical properties were measured at 6, 12 and 18 weeks after rupture using radiostereometry (RSA), which generated data for Young's modulus of elasticity. The mean value of these three measurements was used for analysis. The change in distance between the tantalum beads from 6 to 18 weeks was used as a measure of elongation of the healing tendon callus. Tendon cross-sectional area was determined by ultrasonography at the same time points, again the mean value of the three measurements was used for analyses. The relations between MMPs and TIMPs at three years and mechanical parameters during the early phase of healing were evaluated by calculating Spearman's correlation coefficients.



**Figure 5 a. 3D CT scan at 12 weeks after tendon rupture.** The tantalum markers are enlarged due to artefacts.



**Figure 5 b. RSA examination**

## LABORATORY METHODS

### Achilles tendon transection model (I)

#### Surgical procedure

The animals were anaesthetised with isoflurane gas and given preoperative subcutaneous injections of trimetoprim-sulfadoxine and buprenorphine. The skin on the left hind paw was shaved and washed with chlorhexidine. A skin incision was made over the lateral side of the Achilles tendon. The plantaris tendon was removed. Thereafter, the Achilles tendon was cut transversely 3 mm proximal to the calcaneal insertion. The skin was sutured. Animals were allowed free cage activity immediately after the operation.

#### Mechanical testing

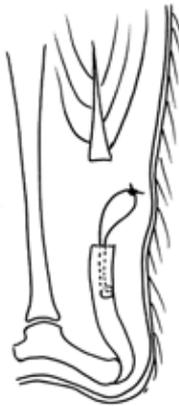
Directly following killing by CO<sub>2</sub> asphyxiation, the tendon with the attaching calcaneus was transected free from other tissues and removed. The callus diameter was measured sagittally and transversely with a digital calliper, and the cross-sectional area was calculated assuming elliptical geometry. The tendon was then fixed between two clamps, one of them a custom made calcaneal clamp holding the bone in 30° dorsiflexion relative to the direction of traction and the other sandwiching the tendon's proximal end between fine sand papers. The clamps were attached

to a materials testing machine (100 R; DDL Inc., Eden Prairie, USA) and pulled at a constant speed of 0.1 mm/s until failure. Peak force, stiffness and energy uptake at 10 % drop of the curve were recorded.

### Achilles tendon suture model (II)

#### Surgical procedure

Animals were anaesthetised with isoflurane gas and given preoperative subcutaneous injections of trimetoprim-sulfadoxine and buprenorphine. The skin was shaved and washed with chlorhexidine. A skin incision was made over the left Achilles tendon. The tendon was dissected free and the suture was inserted into the intact tendon to make a modified (one-sided) Kessler stitch spanning 1 cm longitudinally, starting at the tendon's proximal end 2 mm from the musculotendinous junction. Thereafter, the free ends of the thread were approximated with a double knot, leaving a 1 cm free loop for attachment during pull-out testing. The Achilles tendon was then cut transversely just proximally to the suture to unload the tendon. Thus, the Kessler stitch was only inserted into the distal portion of the cut Achilles tendon to specifically evaluate suture holding capacity (Figure 6). The plantaris tendon was cut and the skin was sutured. Animals were allowed free cage activity immediately after surgery.



**Figure 6. Achilles tendon suture model.** Using the Kessler technique, the suture was inserted only into the distal portion of the cut Achilles tendon to specifically evaluate suture holding capacity. A free loop was left for attachment during mechanical testing

### **Mechanical testing**

After euthanasia, the tendon with the attaching calcaneus was removed and dissected clean from surrounding tissue. The calcaneus was fixed in a custom made clamp, while the suture loop was attached to a hook via a freely movable metal device to allow a straight pull. The complex was mounted in a materials testing machine (100 R) and pulled at a constant speed of 0.1 mm/s until pull-out. Peak force and energy at 10 % drop of the curve were recorded.

### **Anastomosis healing model (III)**

#### **Surgical procedure**

Anaesthesia was induced by a subcutaneous injection of a mixture of fentanyl citrate, droperidol and midazolam. After laparotomy, a standardised 10 mm segment of the colon was resected 6 cm proximally to the anal orifice. An end-to-end anastomosis was constructed using 8 interrupted sutures placed approximately 2 mm from the resection margin. The abdomen was closed with continuous polyglactin suture in the musculofascial layer and metal clips in the skin. The animals were given carprofen for analgesia, and allowed immediate mobilisation.

#### **Mechanical testing**

After euthanasia, the abdomen was opened and the colon freed from adhesions. A 4 cm segment of the colon was resected and cleaned of faecal contents. A corresponding segment was resected in the unoperated rats. The segments were mounted in a materials testing machine (LF Plus; Lloyds Instruments, Fareham, UK) with 10 mm between the clamps, and stretched at a constant speed of 10 mm/min until rupture, recording the maximal load (breaking strength) and the area under the curve to the breaking point (energy uptake).

### **Suture coating (II, III)**

Sterile 3-0 (study II) or 6-0 (study III) polybutester monofilament sutures (Novafil; Tyco Healthcare, Schaffhausen, Switzerland) were activated during 10 seconds on

each side in a radio frequency plasma chamber (Plasmaprep 100; Nanotech, Sweden). Thus, the sutures were exposed to a reactive gas plasma containing free electrons, gas radicals and ions. This causes the surface polymer chains to become cleaved to shorter units, ionised, and radicalised, i.e. chemically activated. The activated sutures were incubated for 30 min in 6 % glutaraldehyde in phosphate buffered saline (PBS) at pH 9. The surfaces were extensively rinsed in PBS at pH 9. Ten layers of fibrinogen (Hyphen BioMed, Neuville-sur-Oise, France) were prepared as follows<sup>121</sup>: the glutaraldehyde-coated sutures were incubated for 30 min in 1 mg/ml fibrinogen dissolved in PBS at pH 7.4. The sutures were extensively rinsed in PBS followed by incubation during 30 min in PBS, pH 5.5, containing 0.2 M ethyl-dimethyl-aminopropylcarbodiimide (EDC; Sigma-Aldrich) and 0.05 M N-hydroxysuccinimide (NHS; Sigma-Aldrich). Then a new 1 mg/ml fibrinogen solution was prepared in PBS buffer, pH 5.5, and the sutures incubated for 30 min in this, rinsed in PBS buffer, and again incubated in the EDC/NHS solution. As the EDC solution is unstable at room conditions, new solutions were prepared every second hour. This procedure was repeated until approximately 10 fibrinogen layers were immobilised. The crosslinked fibrinogen surface was subsequently incubated in EDC/NHS as above, and for 3 hours in a 1 mg/ml doxycycline hyclate solution or for 3 hours in PBS (carrier-coated control sutures), and finally rinsed in distilled water.

Thicknesses of the fibrinogen and doxycycline layers on the sutures were measured by null ellipsometry (Auto-Ell III; Rudolph Research, Flanders, NJ, USA) on a reference silicone surface in air, calculated according to the McCrackin evaluation algorithm<sup>122</sup> and converted into an approximate adsorbed amount per unit area by de Feijter's formula.<sup>123</sup> The assumed refractive index of the protein and immobilised doxycycline film was  $n_f =$

1.465.<sup>124</sup> During the measurements, a 1-nm-thick layer of adsorbed proteins was equivalent to approximately 120 ng/cm<sup>2</sup>.<sup>125</sup>

In study II, the sutures were stored at room temperature in dark in a 1 mg/ml doxycycline PBS solution (pH 5.5), until use. Fibrinogen-coated control sutures were stored in PBS (pH 5.5) under identical conditions. In study III, the sutures were stored in 0.5 mg/ml doxycycline PBS solution or PBS solution only for experiment A. In experiment B, the sutures were dried using nitrogen gas, and stored in sterile bags.

#### **Doxycycline concentration and doxycycline release from the suture surface (I, II)**

In study I, five doxycycline-treated rats in the 14 days group were randomly selected shortly before killing. Rats were anaesthetised with isoflurane gas and blood was collected by cardiac puncture. Blood samples were centrifuged at 2000 x g for 10 min and the serum stored at -70° C until testing. In study II, doxycycline-coated sutures were immersed in sterile NaCl solution. Doxycycline concentrations in the solution at 24 and 72 hours were determined. Measurements were made in triplicates. Results are expressed as percentage of the expected total amount of doxycycline on the thread. The total amount was calculated from ellipsometric measurements on reference silicon surfaces. Concentrations of doxycycline were determined by way of an agar well diffusion assay using *Bacillus cereus* ATCC 11778 as the test organism (Smittskyddsinstitutet, Solna, Sweden)<sup>126</sup>.

#### **Analysis of MMPs and TIMPs (IV)**

Concentrations of MMP-1, -2, -3, -7, -8, -9 and -13 were determined by a particle-based flow-cytometric assay using Fluorokine Multi Analyte Profiling (F-MAP) kits (R&D systems, Minneapolis, USA) in a Luminex 100 Bioanalyzer (Luminex Corp., Austin, USA). These assays recognise proforms, active forms and TIMP-complexed forms of the respective MMPs. The multiplex technology utilises antibody-coated microspheres labelled with fluorescent dyes of different intensities. After reaction with patient samples, the fluorescence emission from the microspheres is quantified in an instrument similar to a flow cytometer. Typically, fifty microspheres per data point are analysed and the median value logged by the software. There is strong correlation between conventional enzyme-linked immunosorbent assay (ELISA) and multiplex F-MAP measurements.<sup>127</sup> The kits have <0.5 % cross-reactivity between MMP species analysed in this study, and the intra-assay coefficient of variation is 5-10 % (data from manufacturer). Concentrations of TIMP-1 and -2 were analysed using sandwich ELISA kits (Quantikine; R&D systems). The TIMP-1 kit recognises free TIMP-1, and TIMP-1 bound to pro-MMP-9 and, to some extent, TIMP-1 bound to active MMPs. The TIMP-2 kit recognises free TIMP-2, TIMP-2 bound to pro-MMP-2 and TIMP-2 bound to active MMPs. All assays were performed according to the manufacturer's instructions. Analyses were performed at Clinical Immunology, Sahlgrenska University Hospital, Göteborg, Sweden.



## RESULTS IN BRIEF

### Study I: Doxycycline impairs tendon repair in rats.

Force at failure and energy uptake were significantly decreased in doxycycline-treated rat Achilles tendons, as compared to untreated controls (Table 4). Doxycycline serum concentration was 3.4 SD 1.0 mikrog/ml.

	5 days		Decrease (%)	8 days		Decrease (%)	14 days		Decrease (%)	p-value
	Mean	SD		Mean	SD		Mean	SD		
<b>Force (N)</b>										
Control	12.8	2.7		23.6	2.6		47.3	8.1		
Doxy	9.8	3.1	23.1	20.2	5.4	14.5	41.5	9.6	12.2	0.003
<b>Stiffness (N/mm)</b>										
Control	5.0	1.2		7.3	1.8		14.9	2.8		
Doxy	4.2	1.3	16.3	6.2	1.5	14.4	15.4	3.0	-3.0	0.36
<b>Energy (Nmm)</b>										
Control	24.8	6.4		65.5	10.0		128.9	37.7		
Doxy	18.9	6.1	23.9	50.8	11.7	22.5	92.6	27.7	28.2*	0.0005
<b>Area (mm<sup>2</sup>)</b>										
Control	7.5	1.7		4.9	1.7		9.6	1.2		
Doxy	6.3	1.4	12.5	4.7	1.4	2.5	8.4	1.6	12.0	0.18
<b>Stress (MPa)</b>										
Control	1.8	0.5		5.5	2.0		5.0	1.3		
Doxy	1.5	0.5	12.9	4.6	1.6	16.8	5.0	1.3	0.0	0.20

**Table 4. Biomechanical evaluation of Achilles tendons in rats treated with systemic doxycycline.**

p-values (two-way ANOVA) for the effects of treatment are based on ln-transformed values. \*P < 0.05 for Bonferroni post hoc test for the effect of treatment. Other post-hoc comparisons for the effect of treatment of force and energy had p > 0.05. P-values for the effect of time were <0.0001 for all comparisons. There were no significant interactions between the effects of treatment and time.

## Study II: Doxycycline-coated sutures improve the suture-holding capacity of the rat Achilles tendon.

Systemic doxycycline treatment and doxycycline-coated sutures improved the suture-holding capacity of rat Achilles tendons (Table 5 and Figure 7). In vitro, most of the doxycycline was released from the sutures after 72 hours (Figure 8).

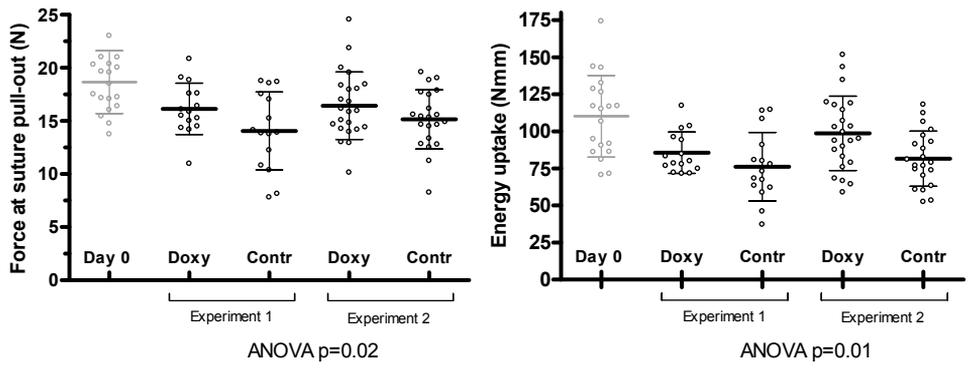
### Force at suture pull-out (N)

Day	Control mean (SD)	n	Doxycycline mean (SD)	n	Difference			p-value
					(95% CI)			
					mean	min	max	
3 <sup>a</sup>	12.6 (3.6)	9	15.0 (4.3)	9	2.4	-1.3	6.0	
3	14.8 (2.0)	9	17.6 (4.1)	8	2.8	-0.4	5.9	
5	16.7 (4.2)	9	14.8 (4.0)	9	-1.9	-5.6	1.9	0.08*, 0.1 <sup>#</sup>
7	15.4 (3.8)	9	18.6 (4.2)	8	3.2	-0.6	7.0	

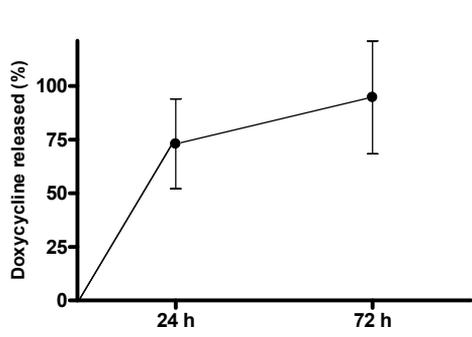
### Energy uptake (Nmm)

Day	Control mean (SD)	n	Doxycycline mean (SD)	n	Difference			p-value
					(95% CI)			
					mean	min	max	
3 <sup>a</sup>	59.5 (19)	9	88.0 (36)	9	29	2.0	55	
3	85.3 (17)	9	105.4 (21)	8	20	1.7	38	
5	98.8 (30)	9	80.7 (24)	9	-18	-43	6.8	0.04*, 0.06 <sup>#</sup>
7	85.6 (23)	9	123.0 (70)	8	37	-13	88	

**Table 5. Systemic doxycycline treatment.** Force (N) at suture pull-out and energy uptake (Nmm) 3, 5 and 7 days after tendon suture. One 3 day-group was operated on at a separate occasion (a). P-values refer to two-way ANOVA for the effect of \*treatment and #time.



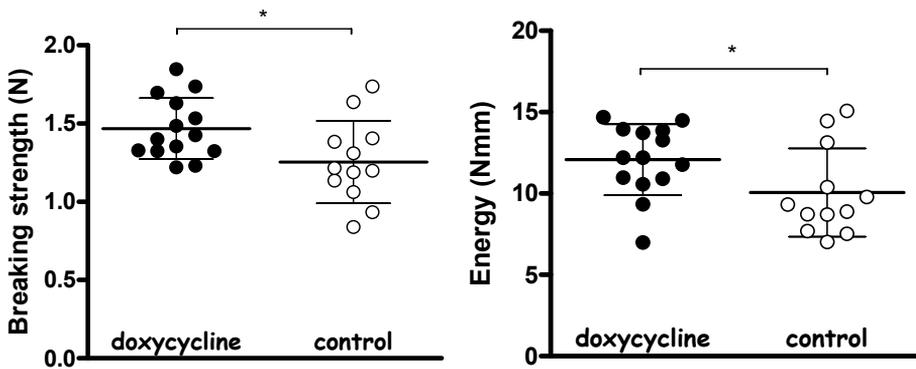
**Figure 7. Local doxycycline treatment using drug-coated sutures.** Force (N) at suture pull-out and energy uptake (Nmm) three days after tendon suture. The p-values refer to the effect of treatment in a two-way ANOVA. The second factor in the ANOVA model was experiment 1 vs experiment 2 (not significant). Controls were uncoated in experiment 1 and carrier (fibrinogen)-coated in experiment 2. Day 0 values (grey) are shown as a reference. Graphs show individual values, means and SDs.



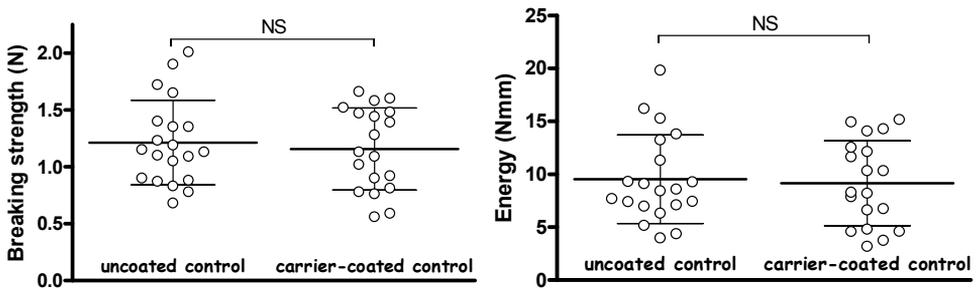
**Figure 8. In vitro drug release from doxycycline-coated sutures.** The percentage released was calculated from the expected total amount of doxycycline on the thread, based on measurements using silicone surfaces. Mean and SD.

# Study III: Doxycycline-coated sutures improve mechanical strength of intestinal anastomoses.

Doxycycline-coated sutures improved the mechanical strength of rat intestinal anastomoses at three days postoperatively (Figure 9). In a second experiment, there was no difference between carrier-coated sutures and uncoated sutures (Figure 10).



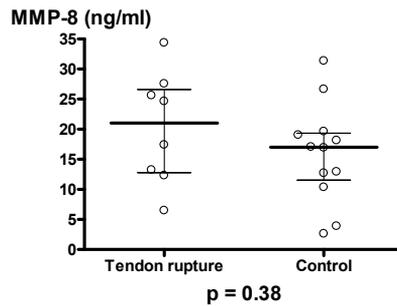
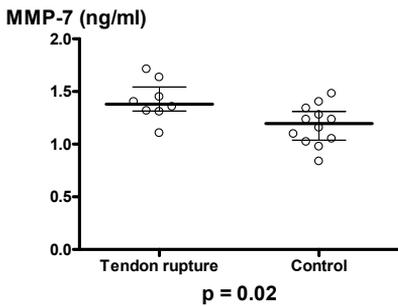
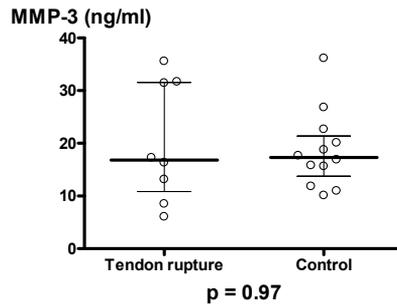
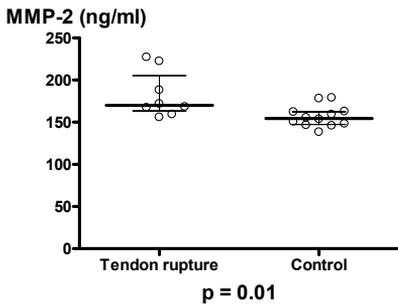
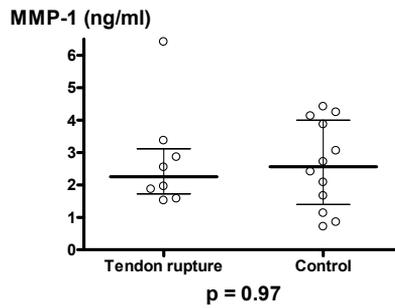
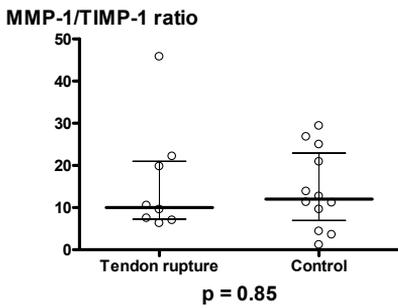
**Figure 9. Anastomotic strength of the rat colon on the third postoperative day in experiment A.** Doxycycline-coated sutures increased the breaking strength (a) by 17 % ( $P=0.026$ ) and the energy uptake at failure (b) by 20 % ( $P=0.047$ ) compared with carrier-coated sutures. Data are shown as mean (thick horizontal line) and SD interval. Filled circles, doxycycline-coated sutures; open circles, carrier-coated sutures. \* $P<0.05$ .

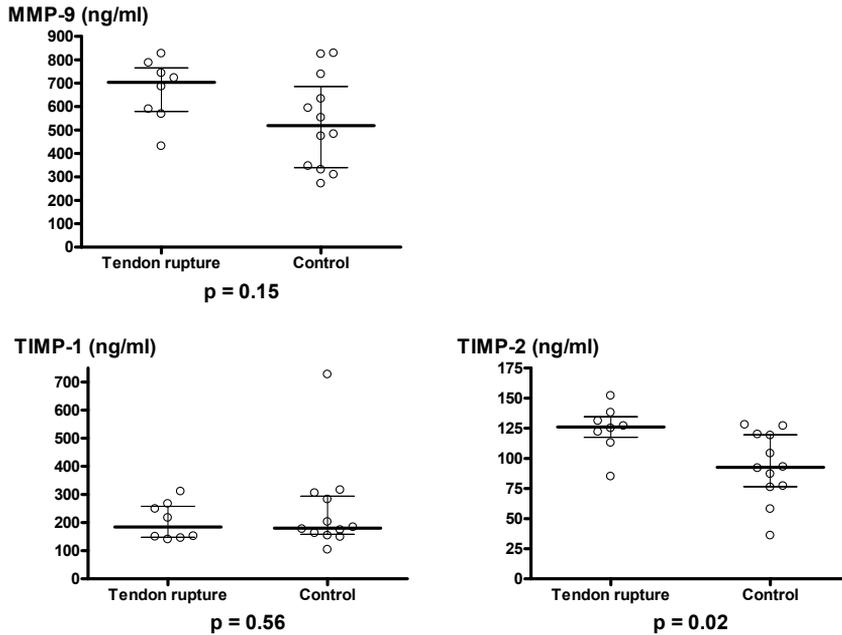


**Figure 10. Anastomotic strength of the rat colon on the third postoperative day in experiment B.** Uncoated and carrier-coated sutures did not differ in breaking strength ( $P=0.64$ ) or energy uptake at failure ( $P=0.78$ ). Data are shown as mean (thick horizontal line) and SD interval. NS; non significant.

# Study IV: Elevation of systemic MMP-2 and -7 and TIMP-2 in patients with a history of Achilles tendon rupture.

MMP-2 and -7, and TIMP-2 were elevated in patients (n=8) who had suffered Achilles tendon rupture more than three years earlier, as compared to control blood donors (n=12; Figure 11). MMP-7 had inverse correlation to modulus of elasticity, a trend towards positive correlation to tendon cross-sectional area and positive correlation to tendon elongation (Table 6). MMP-13 could not be detected in any samples, at a detection limit of 0.052 ng/ml.





**Figure 11. Serum MMPs and TIMPs in patients with a history of Achilles tendon rupture.**

Comparison between patients (n=8) who have suffered Achilles tendon rupture more than three years ago and control blood donors (n=12). MMP assays measure pro-MMPs, active MMPs and TIMP-bound MMPs. Data analysed by two-tailed Mann Whitney U tests. Graphs show individual values, medians and interquartile range.

	Modulus of elasticity		Cross-sectional area		Elongation	
	$r_s$	p-value	$r_s$	p-value	$r_s$	p-value
<b>MMP-1/TIMP-1 ratio</b>	0.21	0.62	-0.07	0.88	0.26	0.54
<b>MMP-2</b>	-0.17	0.70	0.19	0.66	0.26	0.54
<b>MMP-7</b>	-0.83	0.02	0.67	0.08	0.74	0.05
<b>TIMP-2</b>	0.05	0.93	0.19	0.66	-0.24	0.58

**Table 6. Correlation between serum MMPs/TIMPs and early mechanical parameters of tendon healing.**

Serum samples were collected more than three years after rupture to obtain baseline levels of MMPs and TIMPs. Modulus of elasticity, measured by radiostereometric analysis at different loading conditions, and cross-sectional area, measured by ultrasonography, are represented by the average value of measurements at 6, 12 and 18 weeks after injury. Tendon elongation was defined as change in distance between implanted metal markers from 6 to 18 weeks after rupture, as determined by radiography. The MMP-1/TIMP-1 ratio was prespecified as the primary outcome variable in these analyses while MMP-2, MMP-7 and TIMP-2 were explorative, picked for correlation analyses after obtaining results from the group comparisons.  $r_s$ : Spearman's correlation coefficient.

# DISCUSSION

## STUDY I

### Comments on methodology and design

The scope of this study is limited to evaluating the effect of doxycycline on mechanical parameters of the tendon during healing. Doxycycline has other non-antimicrobial effects than MMP-inhibition, i.e. anti-inflammatory properties and inhibition of angiogenesis.<sup>94, 99</sup> Although MMP-inhibition is considered the main non-antimicrobial effect of doxycycline, it was not possible to firmly conclude that the effect of doxycycline on tendon healing was mediated via MMP-inhibition based upon the current study design. The conclusion that a general MMP-inhibitor affects tendon healing could instead have been reached by studying the effect of a drug that inhibits MMPs more specifically or by measuring MMP activity/levels.

The administration of doxycycline in the drinking water was subject to some variation (CV ~20 %), especially since we housed two rats per cage. It was not possible to administer doxycycline by subcutaneous injections since this leads to skin necrosis, and intraperitoneal injections would be expected to cause similar effects. We made a pilot study where we fed rats with doxycycline *per os* (each rat individually using a syringe), and although this did bring us closer to the animals and facilitated handling, there was quite some spillage. Since this once again led to variation (not known how large), we considered it more practical to administer doxycycline in the drinking water.

The time points 5, 8 and 14 days are used as a standard for this model in our laboratory, and represent the different phases of tendon healing (inflammatory, proliferative and early remodelling phases, respectively). I think that this setup is relevant, but for every pair of groups added, one loses statistical power for the post-hoc tests. In our

particular example it might have been wiser to focus on two time points (e.g. 8 and 14 days) and increase the number of animals in each group (without increasing the total number of animals) to decrease the risk of type II error.

My conclusion is that the main limitation of this study is that we did not measure MMP activity, which precluded the possibility of firm conclusions. If we were to repeat the study, I would have suggested measuring MMP activity and using larger groups. The MMP measurements, although based on limited evidence, should probably primarily aim at MMP-1, because it is the MMP most strongly upregulated in human tendon rupture,<sup>32</sup> and MMP-13, since it is upregulated in rotator cuff rupture and is implicated in tendon degradation caused by stress-deprivation.<sup>108, 128</sup> MMP-9 could also be added, since it appears to be an important determinant of healing in several tissues.<sup>90, 91</sup>

### Comments on the findings

The study showed that the general MMP-inhibitor doxycycline impaired healing of rat tendons. Doxycycline serum concentration was similar to the one obtained by administering 100 mg twice a day to humans.<sup>129</sup> This dosage has shown clinical effects for conditions such as rheumatoid arthritis and periodontitis.<sup>114, 130</sup> It should, however, be noted that the concentration of doxycycline in tendon tissue three hours after a single infusion of 200 mg corresponds to 15-25 % of the concentration in serum, in comparison to levels in e.g. intestines, which are equal to, or above, serum levels.<sup>131, 132</sup> Although not steady-state levels, these data indicate that drug concentrations in (injured) tendons might be low. On the other hand, the well perfused tendon regenerates might have concentrations more similar to serum. Furthermore, the subantimicrobial dose of 20 mg

twice a day also has significant clinical effects on rheumatoid arthritis and periodontitis, and is able to inhibit at least some of the MMPs.<sup>103, 114, 130</sup>

Our results suggest that MMPs are important for tendon healing and that doxycycline can be used as a model drug for the study of effects of MMP-inhibitors on tendons. However, they do not rule out the possibility that MMP-inhibitors could be used to enhance tendon healing. This is for several reasons. Firstly, studies of specific MMPs in action during tendon healing might identify those that are deleterious to healing, allowing treatment with inhibitors specific for certain subspecies of the MMPs. Our results from study IV suggest that MMP-7

might be one of these. Secondly, general inhibition of MMPs might show differing effects on tendons depending on the time point(s) of administration during healing, as has been shown for COX-2-inhibitors.<sup>133</sup>

Finally, MMP-inhibitors have been shown to attenuate the decrease in mechanical properties induced by stress-deprivation in vitro and to enhance tendon to bone healing in a rabbit model,<sup>108, 134</sup> which underlines that there are several different orthopaedic applications where MMP-inhibitors might come in use. Thus, the conclusions from this study are that (1) doxycycline has effects (of any kind) on tendons and (2) doxycycline impairs tendon healing, suggesting an important role for MMPs during healing.

## STUDY II

### Comments on methodology and design

A main problem is that the maximum decrease in suture-holding capacity in this model is about 30 %. We have tested several approaches, including addition of inflammatory substances such as carrageenan and prostaglandin E<sub>1</sub>, without further weakening of the suture-holding capacity (unpublished results). We have also done a pilot study in rabbits, and again we had only a 30 % decrease in suture-holding capacity at one week postoperatively (unpublished results). This is surprising, since another group reports 70 % decrease at the same time point in an almost identical model.<sup>73</sup> With a small decrease there is not much room for improvement with pharmacological agents. To increase statistical power, we therefore had to use main results from the two-way ANOVAs (without post-hoc tests), thus basically comparing the entire doxycycline group versus the control group (3, 5 and 7 days grouped in the systemic treatment experiment and the two different subexperiments grouped in the local treatment experiment).

We did not study time points later than seven days because the formation of the tendon callus hindered specific mechanical measurements of suture fixation. Day three was chosen for the local treatment group, since we knew from the systemic treatment experiments that suture-holding capacity did not decrease further after three days. Although it is unclear whether this early time point is clinically relevant, it may be speculated that early improvement in suture-holding capacity will be sustained, thus hypothetically allowing patients to commence physiotherapy at an earlier time point.

### Comments on the findings

This study showed that a minute dose of the MMP-inhibitor doxycycline slightly improved tendon suture fixation. Pharmacological improvement of suture fixation could have important implications in hand surgery and orthopaedics. A stronger suture construct would allow earlier mobilisation. In hand surgery, this has the potential to prevent complications, since it is known from experimental studies that early mobilisation reduces the incidence of adhesions.<sup>135</sup> The positive effects on suture fixation in our study were petite. The negative effects of doxycycline on tendon healing in general, observed in study I, might have been in action in the suture-fixation model as well, thus possibly antagonising the positive effects. More knowledge on activity of specific MMPs around the sutures is required to fully appreciate whether use of (selective) MMP-inhibitors is a feasible approach to improve suture fixation.

Our study shows that suture fixation can be manipulated by local treatment, which supports findings from studies administering growth factors via sutures (see below). In a broader perspective, our study also underlines that biocompatibility of medical materials may be improved by pharmacological agents that inhibit tissue breakdown, as shown for bisphosphonates in fixation of bone implants.<sup>136-138</sup> This approach could have implications for various applications, e.g. mesh surgery in hernia repair.<sup>139</sup>

How can doxycycline lead to negative effects on tendons in study I but positive effects in study II? Study II was designed to specifically evaluate tendon suture fixation. Study I evaluated tendon regenerate formation. Thus, the studies addressed different questions.

### STUDY III

#### Comments on methodology and design

We did not address the mechanisms of action of the sutures in this study. This is particularly important in view of the fact that the bowel is full of bacteria, which could possibly influence anastomotic healing, and which of course could be influenced by doxycycline. Future studies will have to circumvent this by using non-antimicrobial MMP-inhibitors. To start with, GM 6001 seems a good choice, since it is the most efficacious MMP-inhibitor delivered by the systemic route in this model.<sup>82</sup> Another approach might be to use bisphosphonates, which possess potent MMP-inhibitory properties, and are known to work with the coating method.<sup>106, 136</sup>

We chose to focus our study on the third postoperative day, because previous investigations had shown that anastomotic strength is at its lowest at this time point.<sup>83, 87</sup> Although day 7 may not be relevant because the anastomosis can be considered as already healed (similar breaking strength to uninjured colon), studies of other time points such as day 1 and 5 would have added additional information. I suggest that further studies with more potent and specific MMP-inhibitors should include a full time series (1, 3, 5 and possibly 7 days). An inhibitor aiming at MMP-8 and -9, which are upregulated in the direct vicinity of the sutures and have synergistic collagenolytic effects,<sup>84</sup> may prove to be particularly useful.

Breaking strength and energy uptake were used as biomechanical variables, acquired by pulling the anastomosis longitudinally until it breaks. These variables are thought to represent the strength of the anastomosis, based on the notion that the strength is highly dependent on the extracellular matrix holding the sutures. There is some controversy as to whether this type of biomechanical measurement is sensitive enough for the study of early anastomotic healing.<sup>140, 141</sup> In addition, one may question

whether breaking strength is representative of the physiological load placed upon the anastomosis. Some researchers argue that bursting pressure (filling the anastomosis with fluid until it breaks and recording the pressure) is more representative of the physiological situation. Others report both breaking strength and bursting pressure. On the other hand, the effects of MMP inhibitors appear to be at least as pronounced in bursting pressure as in breaking strength measurements (Table 7). Since we did not have access to bursting pressure measurement equipment, we were quite content to be able to show a positive effect with breaking strength measurements only.

To better evaluate the magnitude of the effect of the doxycycline-coated sutures, we could have considered adding another experimental group with doxycycline administered systemically. In our current experimental setup, we were merely able to do (non-statistical) comparisons to historical data from other studies (see below).

#### Comments on the findings

We showed that breaking strength and energy uptake of colonic anastomoses were improved by approximately 20 % in the doxycycline-coated sutures group. There was no statistically significant difference between carrier-coated (fibrinogen) and uncoated sutures, suggesting that the carrier itself has no effects on anastomotic strength. The relative improvement of mechanical parameters with local doxycycline treatment was similar in magnitude to the improvement achieved by systemic doxycycline in a previous study.<sup>88</sup> Several animal studies have consistently shown that anastomotic strength during the early and sensitive postoperative period can be improved with MMP-inhibitors (Table 7). This, of course, leads to speculations whether such treatment might have effects in humans. In 1989, British investigators published a randomised study of approximately 200 patients in whom anastomotic leakage after construction of colonic and rectal anastomoses was

investigated.<sup>89</sup> The effect of the general protease inhibitor, aprotinin, administered intravenously, was studied. This drug had been promising in animal models. In the patients, there was no effect on the composite endpoint, overall anastomotic leakage, but the authors report a higher rate of leakage in the colonic anastomosis subgroup and a lower rate of leakage in the rectal anastomosis group. This not only provides an example of the difficulty of transferring animal data to humans, but also underlines the importance of understanding the pathophysiologic process in humans in order to identify specific molecules to be targeted pharmacologically to try and decrease the risk of leakage. This needs to be addressed in future studies.

In this study we strengthen the conclusion from study II that pharmacological agents can be delivered on the suture surface in minimal doses and improve tissue integrity. Systemic administration of specific and potent novel MMP-inhibitors to humans results in musculoskeletal side effects, such as arthralgias and Dupuytren's contracture-

and frozen shoulder-like conditions, in a considerable proportion of patients.<sup>142, 143</sup> This underlines the important role of MMPs in connective tissue homeostasis. It also shows that administration of the drug on the suture is a clinically relevant approach, which probably would circumvent problems with side effects since systemic doses are negligible. On the other hand, in the clinical postoperative setting after colorectal surgery the duration of MMP-inhibitor therapy may be expected to last for a week or so. It is far from certain that side effects from systemic therapy would be any problem in such a short time period.<sup>105</sup> Nonetheless, direct comparisons between local and systemic drug delivery need further study, as this will give information on which form of therapy would be most efficacious.

In addition to MMP-inhibitors, several growth factors and growth factor preparations have been shown to improve anastomotic healing,<sup>144-146</sup> and local administration by way of the suture might provide an effective means of drug administration to where it is needed, i.e. the suture line.

<b>MMPI</b> (daily dose; route of administration)	<b>Part of intestine</b>	<b>Increase in breaking strength by MMPI</b>	<b>Increase in bursting pressure by MMPI</b>
<b>BB-1101</b> <sup>87</sup> (30 mg/kg; s.c.)	Colon	48 %	NA
<b>BE-16627B</b> <sup>147</sup> (10 mg/kg; s.c.)	Colon	NA	28 %
<b>BB-94</b> <sup>148</sup> (40 mg/kg; i.p.)	Ileum	NA	none
<b>BB-94</b> <sup>149</sup> (30 mg/kg; i.p.)	Ileum	108 %	58 %
	Colon	27 %	54 %
<b>Doxycycline</b> * <sup>88</sup> (40 mg/kg; p.o.)  (40 mg/kg; s.c.)	Ileum	100 %	none
	Colon	none	36 %
	Ileum	80 %	none
<b>GM 6001</b> <sup>82</sup> (100 mg/kg; s.c.)	Colon	25 %	100 %
	Colon	99 %	NA
<b>Doxycycline</b> (coated sutures; III)	Colon	17 %	NA

**Table 7. Relative effects of MMP-inhibitors (MMPIs) on mechanical properties of the healing bowel in the rat anastomosis model on postoperative day 3 or 4.** Studies in order of publication date.

\* approximate per cent values – exact mean/median data not fully provided in the original article. p.o.: per oral. s.c.: subcutaneous. i.p.: intraperitoneal. NA: non applicable. GM6001: ilomostat. BB-94: batimastat.

### Comments concerning drug-coated sutures (II and III)

We used a suture coating procedure that immobilises multiple layers of fibrinogen onto the suture surface, with drug bound into this fibrinogen film. The method is simple and relatively cheap, but it is probably more cumbersome than some of the dip-coating techniques developed recently. For the coating method to have clinical potential, there is need for further studies related to the coating procedure,

such as improvement in efficacy, rapidity and cost of the coating and factors related to possibility of commercial production. Although we did study the release of doxycycline from the suture in vitro, the experiments could have been extended by measuring doxycycline release from coated sutures that had been placed in animal tendons for e.g. 24 and 72 hours.

Only minute doses of the drug were delivered by way of the suture. In study II, about 30 ng of doxycycline were delivered

by one centimetre of the suture thread (size 3-0), while approximately 7 ng per centimetre suture (size 6-0) were delivered in study III. We do not know whether these doses are enough to inhibit a relevant proportion of MMPs acting around the sutures, and if they do, at which distance from the sutures they affect MMPs. This could have been addressed by measuring local MMP activity or collagen breakdown.<sup>150, 151</sup> Neither do we know where the drug is deposited. This issue could be addressed by utilising the fluorescent properties of doxycycline.

Although our studies are the first to administer inhibitors of tissue breakdown

via the suture surface, a couple of other studies report growth factor-coating of suture material and successful administration in tendon repair (Table 8). This could open up for further studies evaluating local administration of drugs in orthopaedics. Local drug delivery is already widely applied in several medical disciplines, e.g. drug eluting stents in coronary interventions. In orthopaedics, local drug delivery is used in clinical practice for various indications, e.g. cement supplemented by antibiotics to prevent infection after prosthetic surgery and locally delivered anaesthetics and analgetics to relieve postoperative pain.<sup>152, 153</sup>

Drug	Model	Results
<b>GDF-5</b>	Rat Achilles tendon transection and suture.	GDF-5 improved mechanics at three but not six weeks. Improved histological score with GDF-5 treatment. <sup>154</sup>
<b>bFGF</b>	Rat flexor tendon transection and suture.	bFGF improved mechanics at three but not one or six weeks. bFGF accelerated cellular proliferation. <sup>155</sup>
<b>GDF-5</b>	Rat Achilles tendon transection and suture.	GDF-5 improved mechanics at one, two and four but not eight weeks. Induction of cartilage-like cells. <sup>156</sup>
<b>Collagen</b>	In vitro human tenocyte cell culture.	Type I collagen induced adhesion, proliferation and protein synthesis in tenocytes. <sup>157</sup>
<b>EGF, PDGF, KGF</b>	In vitro human tenocyte cell culture.	Increased proliferation with EGF, to a lesser extent PDGF but not KGF. <sup>158</sup>

**Table 8.** Summary of experimental studies using sutures coated with pharmacological agents to improve tissue healing. GDF: growth and differentiation factor. bFGF: basic fibroblast growth factor. EGF: epidermal growth factor. PDGF: platelet derived growth factor. KGF: keratinocyte growth factor. Studies concerned with suture coating to prevent bacterial infections or to modulate the immune response are omitted.

## STUDY IV

### Comments on methodology and design

There are several factors that might have affected the results in this study. Firstly, it is based on a limited number of patients which meant that we had 70 % power ( $\alpha$  0.05) to detect a 30 % difference in means between groups, assuming standard deviations corresponding to 20 % of the means. This calculation is based on parametric statistics and not fully applicable to our data, but still gives a hint that there was a risk of falsely missing relevant differences (type II error). Secondly, we chose to use controls selected among blood donors. This is a potential source of systematic error, i.e. selection bias, because of the possibility that the blood donors, who are probably more healthy than the general population, could have lower MMP levels, increasing the risk of false positive findings (type I error). It is difficult to find healthy controls at the department or the hospital and I would like to argue that selection of controls our way is reasonable. Although they do not have an equal chance as any other healthy person in the general population to be asked to participate, it is likely that they represent people who would be interested in participating. An alternative approach would be randomly selecting controls from the general population, e.g. via the population register. Thirdly, there is another potential source of systematic error. We had no knowledge of control subjects' history of tendon disease, the presence of which (misclassification bias) would dilute the differences and increase the risk of type II error. Although the chance of anyone of them having had tendon rupture is quite low, tendon problems in general are quite common, and since they all could have similar aetiopathogeneses, patients with these conditions should be excluded or at least identified. A questionnaire concerning tendon and ligaments problems is included in our next study. On the other hand, a main strength of this study was that detailed mechanical measurements of tendon healing

were performed so that a relation between these and MMPs and TIMPs could be studied.

There is some controversy as to whether analysis of MMPs should be performed in plasma or serum. Apparently, serum not only contains MMPs released from tissues but also enzymes released during clotting of thrombocytes and activation of leukocytes in the serum tube during sample preparation. Therefore, some argue that serum levels of MMPs are inaccurate and that plasma always should be used for MMP analyses.<sup>159</sup> Indeed, MMP levels in serum are higher than in plasma. However, other researchers have shown that correlation between serum and plasma MMPs is good for several (but not all) MMPs, as long as sampling procedures are standardised.<sup>160, 161</sup> In addition, one could argue that if one wants to study the full "proteolytic potential" in a patient, one might want to include MMPs also stored in blood cells. On the other hand, if one wants to study MMPs released from tissues, it is probably better to use plasma. We were actually aiming at the former, why serum samples ought to serve best to answer our question. We are however currently recruiting patients to a new study with a similar research question, where we are aiming at measuring MMPs in both serum and plasma.

The MMP assay recognises active MMPs, pro-MMPs and MMPs bound to TIMPs. Several approaches could be used to further study the proteolytic activity in these samples, such as measuring only the active component of specific MMPs, or measuring total collagenolytic and gelatinolytic activity. By applying different methods (that hopefully show the same thing) one could increase the validity of the findings.

Selection of MMP-1/TIMP-1 ratio as the primary outcome variable had a weak basis. The only real evidence behind it was that MMP-1 mRNA was previously shown to be

the most highly upregulated among the MMPs in acute tendon rupture.<sup>32</sup> Further, MMP-1 is capable of degrading intact fibrillar collagens, which means that it is one of the MMPs that controls key limiting steps in degradation of tendon tissue. The available data suggested that TIMP-1 was the only TIMP elevated in acute Achilles tendon rupture.<sup>32</sup> It was necessary for us to have some primary hypothesis (besides “MMPs are elevated”), and MMP-1/TIMP-1 ratio seemed reasonable since it represented one degradative and one protective component.

Data were analysed by separate Mann Whitney U tests and there was no correction for multiple comparisons. This of course increases the risk for false positives, but is a reasonable approach for an exploratory study.<sup>162, 163</sup> We had one primary hypothesis, and this was rejected. Thus, the results should be interpreted in the context of an exploratory study, i.e. they need to be confirmed.

We measured MMPs and TIMPs in patients who had suffered tendon rupture more than three years earlier. This was done to acquire baseline values, based on the assumption that the healing process is finished by three years. Studies of human Achilles tendon lesions created by collecting tendon biopsies suggest that tendons have healed at one year after injury, as assessed by magnetic resonance imaging.<sup>164</sup> On the other hand, histological and ultrastructural studies of patellar tendons suggest that a certain degree of abnormality in the structure of the ECM and increase in cellularity and vascularity is evident even up to 10 years after injury.<sup>165, 166</sup> However, in biopsy material obtained at two years after harvest of the central third of the patellar tendon there is no immunohistochemical staining for type III collagen, strongly suggesting that there is no active healing.<sup>167</sup> These data show that tendon tissue never regains

original composition after injury. Although healing seems absent, it cannot be deduced from these studies whether MMP activity has returned back to normal. If true baseline values are of interest, biobank based research may be more suitable, since it would allow access to preinjury samples. Finally, we have preliminary data (not shown) that suggest that MMP-7 is elevated already at the time when patients are first diagnosed with acute Achilles tendon rupture. Although these results need confirmation, they support the hypothesis that elevation of (at least) MMP-7 is constitutive and not a reflection of changes induced by the rupture itself.

### **Comments on the findings**

The results suggest that people who have suffered Achilles tendon rupture differ from the normal population in their proteolytic capacity. Such differences might be genetic. If one wants to study whether there is possible genetic influences to a disease, there are several other approaches. The classical one is the epidemiological approach to heredity. There are no reports on family history of Achilles tendon rupture in the literature, although one study lends support to a generalised predisposition to tendon rupture.<sup>47</sup> Epidemiologic studies have also shown increased family risk of rotator cuff and anterior cruciate ligament injuries.<sup>48, 49</sup> Another approach could be studies of genetic polymorphisms. Previous reports have shown an association between a polymorphism in the tenascin C gene and tendon rupture.<sup>46</sup> In the field of MMPs, a couple of findings relevant to tissue healing have been reported. There is an association between a polymorphism in the MMP-1 gene and aseptic loosening of hip prostheses.<sup>93</sup> In addition, a polymorphism in the promotor region of the MMP-1 gene was found to be associated with early failure of dental implants.<sup>168</sup>



# SPECULATIONS AND FUTURE RESEARCH

## MMPs IN TENDON RUPTURE AND HEALING

---

Our results from study I, which show that doxycycline impairs mechanical properties of the Achilles tendon during healing, underline that MMPs are important for physiological healing of tendons. Future research in this area should be aimed at studying gene expression, levels and activity of tendon MMPs and TIMPs during healing. Preferably, healing should be studied in models that involve healthy tendons as well as tendinopathic tendons, to delineate whether there is any difference between the two. This might identify MMPs that are deleterious to tendon healing, thus allowing selective inhibition of these MMPs to enhance healing. This type of study is intimately intertwined with issues that have evolved from study IV, which shows that baseline systemic levels of MMP-7 are closely related to mechanical parameters of the tendon during healing. That is, higher MMP-7 levels are associated with lower tendon modulus of elasticity, and larger cross-sectional area and tendon elongation, indicating poorer tissue quality. This indicates that MMP-7 might be a relevant target to improve tendon healing. Hopefully, some day, we will see an MMP-inhibitor in clinical use for tendon disorders.

We showed that MMP-2, MMP-7 and TIMP-2 were elevated in patients with a history of Achilles tendon rupture. This has implications for the aetiopathogenesis of tendon rupture and needs further exploration. Firstly, we plan to study systemic levels of the relevant MMPs and TIMP in patients with acute Achilles tendon rupture. There are then several studies that could be done to further investigate the roles of MMPs and TIMPs in tendon rupture and outcome of tendon healing. The relative

levels of molecular forms of MMPs (e.g. pro- and active forms) should be investigated, as well as polymorphisms in MMP and TIMP genes in patients with tendon rupture.

An epidemiological approach to the aetiology of Achilles tendon rupture has, this far, hardly been applied. These types of studies could provide insight into the hereditary component of tendon disease. With the extensive amount of data available in Scandinavian registries this could be done on a population-based basis. In addition, registries could be used to study connections between diseases with possible common pathobiological features, i.e. conditions where the extracellular matrix is affected. As an example, studies have revealed that alterations of the extracellular matrix have a prominent role in inguinal hernia. Intriguingly, there are findings that suggest that these alterations are generalised. Studies on cultured dermal fibroblasts obtained from skin of patients with primary and recurrent inguinal hernia revealed lowered type I/III collagen ratio, mainly explained by elevated levels of type III collagen.<sup>169, 170</sup> In addition, total and active MMP-2 was elevated in cell cultures from transversalis fascia samples of patients with direct inguinal hernia.<sup>171</sup> Results for MMP-1 and -13 are somewhat inconsistent, but point toward a minor role for these proteinases. In addition, an association between abdominal aortic aneurysm, a disease where the role of MMP-mediated collagen degradation is firmly established,<sup>16</sup> and inguinal hernia, has been reported.<sup>172</sup> Furthermore, alterations in the ratio between type I and III collagens, and elevation of MMPs are associated with anastomotic leakage after colorectal surgery.

Together, this has key implications for the results of study IV, supporting the hypothesis that local manifestations of an altered extracellular matrix might have a generalised background. Thus, there may be a subset of individuals with weaker collagen composition and an elevated capacity for proteolysis. Preoperative identification of these putative individuals may lead to improved peri- and postoperative care.

Is there any clinical utility in measuring systemic MMPs? We showed that baseline MMP-7 was strongly related to mechanical parameters of the healing tendon. Future studies should be directed at investigating the feasibility of measuring systemic MMP in prognostic studies. These will reveal if there is any relationship between MMPs or TIMPs sampled at admission to hospital and final clinical outcome, e.g. at one or three years after tendon rupture. A similar approach has been used in prediction of the disease course in osteoarthritis. Patients with plasma MMP-3 levels in the upper tertile of the baseline distribution were more likely to suffer progression of knee joint

space narrowing during a 30 month period, as compared to patients in the lower tertile (odds ratio 4).<sup>173</sup> Studies of tendon healing could also be directed at investigating whether there is any utility in measuring systemic MMPs at different time points to monitor the healing process. This could provide important information with regard to the intensity of rehabilitation. Studies of patients with long bone fractures suggest that it is possible to monitor the healing process by measuring systemic levels of several molecules thought to be important in repair.<sup>92, 174</sup>

Although study II shows that MMP-inhibitor-coated sutures could serve to improve suture fixation, it is doubtful whether this approach is worth pursuing, since improvement of mechanical strength was very small. One approach could be to test MMP-inhibitors selective for certain MMP-subspecies. Before that, studies on which MMPs to target are needed.

## MMPs IN ANASTOMOTIC LEAKAGE

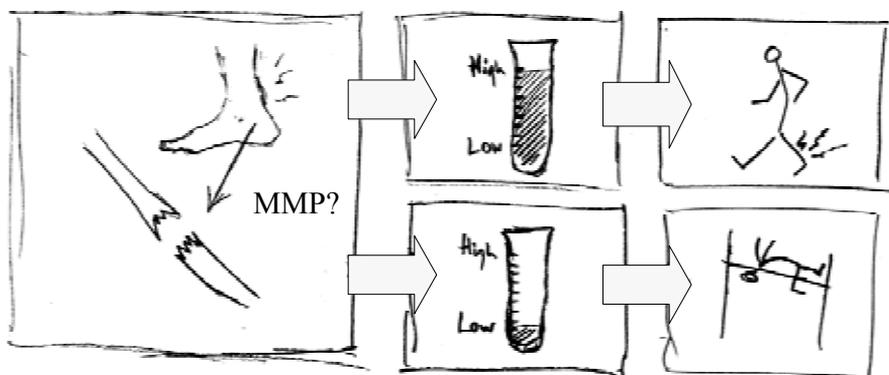
---

Anastomotic leakage is a severe complication after colorectal surgery and there has been a long search for strategies and medications to decrease its incidence. Is prophylactic treatment with MMP-inhibitors the way to go? Our study shows that the strength of experimental anastomoses can be improved by minute doses of an MMP-inhibitor administered via the suture and there is relatively robust experimental evidence that systemic MMP-inhibition improves the strength of the anastomosis. Before starting up clinical trials, the pathophysiology of anastomotic leakage in humans needs further attention. Which particular MMPs are responsible for the

deterioration of the anastomosis? Would inhibition of some of the MMPs even be harmful? Studies of the colorectal MMP response to surgery in patients with and without anastomotic leakage will answer some of these questions and form the basis for selection of an MMP-inhibitor selective for certain species of the MMPs. Short term systemic treatment is probably feasible with regards to side effects.<sup>105</sup> Local treatment could be an option would side effects from systemic therapy be limiting. However, to confirm the feasibility of local treatment, studies in species larger than rats are needed.

## DÅLIGT LÄKKÖTT [dawlicked lairk-shot<sup>§</sup>]

*Dåligt läkkött* is a Swedish expression. *Dåligt* means bad, while *bra* means good. *Läkkött* is a word that is intranslatable to a specific English word or phrase (Nigel Musk, personal communication). *Läkkött* describes, in a somewhat raw, but precise, vitalising and colloquial way, the healing ability of wounds. *Läkkött* literary means healing flesh, and there is *dåligt läkkött* (bad healing ability of the flesh) and *bra läkkött* (good healing ability of the flesh). Every Swedish speaking person knows the meaning of the phrase, and probably everyone has used it sometime; “You know, he has *dåligt läkkött* so he couldn’t play soccer for 11 months after that ankle sprain”. As the reader might have guessed, I love this expression. As any other expression commonly used in the community, it ought to have some degree of truth behind it. Haven’t you wondered; “how’s this one going to heal”; after finishing up the stitches of another operation? In many instances, it would be very useful to know the outcome of healing on beforehand. We should also attempt to prevent complications of tissue healing. Maybe, MMPs are the way to go.



Future scenario?

<sup>§</sup> British English pronunciation



# SAMMANFATTNING PÅ SVENSKA

Kirurger behöver ständigt nya verktyg. Sådana kan vara av plast och stål, men det finns också verktyg att hämta från sjukdomsbiologin. Det har visat sig att proteinnedbrytande enzymer, s.k. metalloproteinaser, har betydelse vid sjukdomar som drabbar bindväven. Våra studier handlar om metalloproteinaser och deras hämmare. Syftet är att förse kirurger med nya verktyg.

Akut bristning av hälsenan drabbar aktiva människor, oftast i samband med idrottsutövning. Skadan tar lång tid att läka, och en andel av patienterna kommer inte tillbaka till tidigare aktivitetsnivå. Tidigare studier har visat att metalloproteinaser har betydelse vid hälseneskador. Vi fann att patienter som tidigare drabbats av hälseneskada hade förhöjda nivåer av metalloproteinas-2 och -7 samt metalloproteinashämmare-2. Det fanns också ett samband mellan metalloproteinas-7 och styrkan i den läkande senan, där högre nivåer var kopplade till sämre styrka. Vår undersökning fann de förhöjda halterna av proteinerna i blodet, något som antyder att det föreligger en generell förändring i förmågan att bryta ned proteiner hos dessa patienter. En sådan förändring skulle kunna vara genetisk. Denna studie öppnar vägen för fortsatta studier av betydelsen av systemiska faktorer för senskador.

Vi har också undersökt hur metalloproteinashämmare påverkar läkningen av hälsenan. I ett experiment fann vi att en brett verkande hämmare som gavs till försöksdjur i dricksvattnet hämmade läkningen av hälsenan. Detta var den första studien som visade att metalloproteinashämmare påverkar senor. Vi visade sedan att en metalloproteinashämmare förbättrar infästningen av kirurgiska sytrådar i hälsenan. Denna effekt sågs både när läkemedlet gavs i dricksvattnet och när vi tillförde det lokalt, fäst på sytrådens yta. Vi kunde i ett annat experiment bekräfta att lokal tillförsel av metalloproteinashämmare på sytråden också fungerade i en modell för grovtarmsläkning, där försöksdjur som fått läkemedlet hade starkare tarmskarv än gruppen som inte fått denna behandling.

Kanske får vi i framtiden se kirurger använda sig av farmakologiska och diagnostiska vertyg baserade på kunskaper om metalloproteinaser.



# ACKNOWLEDGEMENTS

I wish to express my gratitude to all colleagues, co-workers and people I know who have contributed to this thesis, directly or indirectly. In particular, I would like to thank:

Per Aspenberg, for enthusiastic encouragement, creative curiosity, a vivid imagination, some conservatism, and unconditional support.

Anna Missios, for your love and hard work.

Pernilla Eliasson, for your positive attitude.

Mårten Fellenius, for a lot of DIRTY work.

Pentti Tengvall, we are dependent on your knowledge!

Agneta Askendal, for putting up with the entangled sutures.

Anna Fahlgren, for working so hard for all of us.

Olena Virchenko, for nice chats and introduction to laboratory work.

Ali Sodeifi and Bibbi Mårdh, for technical assistance and answers to bizarre questions.

Nigel Musk, for useful comments on *dåligt läkkött*.

Claes Hildebrand, for guiding me into the academic sphere so early on.

Thorsten Schepull and Teréz Hanqvist, for your never-ending efforts in collecting the patients for the Achilles tendon studies.

The staff at the animal department.

Peter Matthiessen, for your open-mindedness for new anastomosis studies.

Magnus S Ågren, for your sound scientific approach.

Magnus Grenegård, for that useful summer in your lab.

Josef Brandström, for your friendship and for kind of leading me into medicine.

Martin Denstedt, for believing in me.

Arvid Engström, for your friendship and fruitful discussions.

Lena Pasternak, mom.

The studies were supported by the strategic research programme Materials in Medicine in Linköping, the Swedish Research Council and Landstinget i Östergötland research funds.



## REFERENCES

1. Sharma P, Maffulli N. Tendon injury and tendinopathy: healing and repair. *J Bone Joint Surg Am.* 2005;87:187-202.
2. Jarvinen TA, Kannus P, Jarvinen TL, et al. Tenascin-C in the pathobiology and healing process of musculoskeletal tissue injury. *Scand J Med Sci Sports.* 2000;10:376-382.
3. Mandal M, Mandal A, Das S, et al. Clinical implications of matrix metalloproteinases. *Mol Cell Biochem.* 2003;252:305-329.
4. Pearce WH, Shively VP. Abdominal aortic aneurysm as a complex multifactorial disease: interactions of polymorphisms of inflammatory genes, features of autoimmunity, and current status of MMPs. *Ann N Y Acad Sci.* 2006;1085:117-132.
5. van Meurs J, van Lent P, Stoop R, et al. Cleavage of aggrecan at the Asn341-Phe342 site coincides with the initiation of collagen damage in murine antigen-induced arthritis: a pivotal role for stromelysin 1 in matrix metalloproteinase activity. *Arthritis Rheum.* 1999;42:2074-2084.
6. Dollery CM, Libby P. Atherosclerosis and proteinase activation. *Cardiovasc Res.* 2006;69:625-635.
7. Hidalgo M, Eckhardt SG. Development of matrix metalloproteinase inhibitors in cancer therapy. *J Natl Cancer Inst.* 2001;93:178-193.
8. Kossakowska AE, Edwards DR, Prusinkiewicz C, et al. Interleukin-6 regulation of matrix metalloproteinase (MMP-2 and MMP-9) and tissue inhibitor of metalloproteinase (TIMP-1) expression in malignant non-Hodgkin's lymphomas. *Blood.* 1999;94:2080-2089.
9. Corps AN, Curry VA, Buttle DJ, et al. Inhibition of interleukin-1beta-stimulated collagenase and stromelysin expression in human tendon fibroblasts by epigallocatechin gallate ester. *Matrix Biol.* 2004;23:163-169.
10. Meller D, Li DQ, Tseng SC. Regulation of collagenase, stromelysin, and gelatinase B in human conjunctival and conjunctivochalasis fibroblasts by interleukin-1beta and tumor necrosis factor-alpha. *Invest Ophthalmol Vis Sci.* 2000;41:2922-2929.
11. Gabison EE, Hoang-Xuan T, Mauviel A, Menashi S. EMMPRIN/CD147, an MMP modulator in cancer, development and tissue repair. *Biochimie.* 2005;87:361-368.
12. Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. *J Cell Sci.* 2002;115:3719-3727.
13. Chirco R, Liu XW, Jung KK, Kim HR. Novel functions of TIMPs in cell signaling. *Cancer Metastasis Rev.* 2006;25:99-113.
14. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res.* 2006;69:562-573.
15. Bramono DS, Richmond JC, Weitzel PP, et al. Matrix metalloproteinases and their clinical applications in orthopaedics. *Clin Orthop Relat Res.* 2004:272-285.
16. Sakalihan N, Limet R, Defawe OD. Abdominal aortic aneurysm. *Lancet.* 2005;365:1577-1589.
17. Martignetti JA, Aqeel AA, Sewairi WA, et al. Mutation of the matrix metalloproteinase 2 gene (MMP2) causes a multicentric osteolysis and arthritis syndrome. *Nat Genet.* 2001;28:261-265.
18. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res.* 2003;92:827-839.
19. Pavlaki M, Zucker S. Matrix metalloproteinase inhibitors (MMPi): the beginning of phase I or the termination of phase III clinical trials. *Cancer Metastasis Rev.* 2003;22:177-203.
20. Illman SA, Lehti K, Keski-Oja J, Lohi J. Epilysin (MMP-28) induces TGF-beta mediated epithelial to mesenchymal transition in lung carcinoma cells. *J Cell Sci.* 2006;119:3856-3865.
21. Chakraborti S, Mandal M, Das S, et al. Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem.* 2003;253:269-285.
22. Foos MJ, Hickox JR, Mansour PG, et al. Expression of matrix metalloproteinase and tissue inhibitor of metalloproteinase genes in human anterior cruciate ligament. *J Orthop Res.* 2001;19:642-649.
23. Arnoczky SP, Lavagnino M, Egerbacher M. The mechanobiological aetiopathogenesis of tendinopathy: is it the over-stimulation or the under-stimulation of tendon cells? *Int J Exp Pathol.* 2007;88:217-226.
24. Rees JD, Wilson AM, Wolman RL. Current concepts in the management of tendon disorders. *Rheumatology (Oxford).* 2006;45:508-521.
25. Astrom M, Rausing A. Chronic Achilles tendinopathy. A survey of surgical and histopathologic findings. *Clin Orthop Relat Res.* 1995:151-164.
26. de Mos M, van El B, DeGroot J, et al. Achilles tendinosis: changes in biochemical composition and collagen turnover rate. *Am J Sports Med.* 2007;35:1549-1556.

27. Wang JH, Jia F, Yang G, et al. Cyclic mechanical stretching of human tendon fibroblasts increases the production of prostaglandin E2 and levels of cyclooxygenase expression: a novel in vitro model study. *Connect Tissue Res.* 2003;44:128-133.
28. Riley G. The pathogenesis of tendinopathy. A molecular perspective. *Rheumatology.* 2004;43:131-142.
29. Riley G. Tendinopathy--from basic science to treatment. *Nat Clin Pract Rheumatol.* 2008;4:82-89.
30. Maffulli N, Khan KM, Puddu G. Overuse tendon conditions: time to change a confusing terminology. *Arthroscopy.* 1998;14:840-843.
31. Maffulli N, Ewen SW, Waterston SW, et al. Tenocytes from ruptured and tendinopathic achilles tendons produce greater quantities of type III collagen than tenocytes from normal achilles tendons. An in vitro model of human tendon healing. *Am J Sports Med.* 2000;28:499-505.
32. Jones GC, Corps AN, Pennington CJ, et al. Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human achilles tendon. *Arthritis Rheum.* 2006;54:832-842.
33. Corps AN, Robinson AH, Movin T, et al. Increased expression of aggrecan and biglycan mRNA in Achilles tendinopathy. *Rheumatology.* 2006;45:291-294.
34. Riley GP, Harrall RL, Cawston TE, et al. Tenascin-C and human tendon degeneration. *Am J Pathol.* 1996;149:933-943.
35. Alfredson H, Ohberg L. Sclerosing injections to areas of neo-vascularisation reduce pain in chronic Achilles tendinopathy: a double-blind randomised controlled trial. *Knee Surg Sports Traumatol Arthrosc.* 2005;13:338-344.
36. van Snellenberg W, Wiley JP, Brunet G. Achilles tendon pain intensity and level of neovascularization in athletes as determined by color Doppler ultrasound. *Scand J Med Sci Sports.* 2007;17:530-534.
37. de Vos RJ, Weir A, Cobben LP, Tol JL. The value of power Doppler ultrasonography in Achilles tendinopathy: a prospective study. *Am J Sports Med.* 2007;35:1696-1701.
38. Alfredson H. The chronic painful Achilles and patellar tendon: research on basic biology and treatment. *Scand J Med Sci Sports.* 2005;15:252-259.
39. Schubert TE, Weidler C, Lerch K, et al. Achilles tendinosis is associated with sprouting of substance P positive nerve fibres. *Ann Rheum Dis.* 2005;64:1083-1086.
40. Cury PR, Canavez F, de Araujo VC, et al. Substance P regulates the expression of matrix metalloproteinases and tissue inhibitors of metalloproteinase in cultured human gingival fibroblasts. *J Periodontal Res.* 2007.
41. Ackermann PW, Li J, Lundeberg T, Kreicbergs A. Neuronal plasticity in relation to nociception and healing of rat achilles tendon. *J Orthop Res.* 2003;21:432-441.
42. Burssens P, Steyaert A, Forsyth R, et al. Exogenously administered substance P and neutral endopeptidase inhibitors stimulate fibroblast proliferation, angiogenesis and collagen organization during Achilles tendon healing. *Foot Ankle Int.* 2005;26:832-839.
43. Steyaert AE, Burssens PJ, Vercruyse CW, et al. The effects of substance P on the biomechanical properties of ruptured rat Achilles' tendon. *Arch Phys Med Rehabil.* 2006;87:254-258.
44. Murrell GA. Using nitric oxide to treat tendinopathy. *Br J Sports Med.* 2007;41:227-231.
45. Mokone GG, Schweltnus MP, Noakes TD, Collins M. The COL5A1 gene and Achilles tendon pathology. *Scand J Med Sci Sports.* 2006;16:19-26.
46. Mokone GG, Gajjar M, September AV, et al. The guanine-thymine dinucleotide repeat polymorphism within the tenascin-C gene is associated with achilles tendon injuries. *Am J Sports Med.* 2005;33:1016-1021.
47. Aroen A, Helgo D, Granlund OG, Bahr R. Contralateral tendon rupture risk is increased in individuals with a previous Achilles tendon rupture. *Scand J Med Sci Sports.* 2004;14:30-33.
48. Flynn RK, Pedersen CL, Birmingham TB, et al. The familial predisposition toward tearing the anterior cruciate ligament: a case control study. *Am J Sports Med.* 2005;33:23-28.
49. Harvie P, Ostlere SJ, Teh J, et al. Genetic influences in the aetiology of tears of the rotator cuff. Sibling risk of a full-thickness tear. *J Bone Joint Surg Br.* 2004;86:696-700.
50. Houshian S, Tscherning T, Riegels-Nielsen P. The epidemiology of Achilles tendon rupture in a Danish county. *Injury.* 1998;29:651-654.
51. Suchak AA, Bostick G, Reid D, et al. The incidence of Achilles tendon ruptures in Edmonton, Canada. *Foot Ankle Int.* 2005;26:932-936.
52. Moller A, Astron M, Westlin N. Increasing incidence of Achilles tendon rupture. *Acta Orthop Scand.* 1996;67:479-481.
53. Leppilahti J, Puranen J, Orava S. Incidence of Achilles tendon rupture. *Acta Orthop Scand.* 1996;67:277-279.
54. Kannus P, Jozsa L. Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. *J Bone Joint Surg Am.* 1991;73:1507-1525.

55. Tallon C, Maffulli N, Ewen SW. Ruptured Achilles tendons are significantly more degenerated than tendinopathic tendons. *Med Sci Sports Exerc.* 2001;33:1983-1990.
56. Khan RJ, Fick D, Keogh A, et al. Treatment of acute achilles tendon ruptures. A meta-analysis of randomized, controlled trials. *J Bone Joint Surg Am.* 2005;87:2202-2210.
57. Kangas J, Pajala A, Ohtonen P, Leppilahti J. Achilles tendon elongation after rupture repair: a randomized comparison of 2 postoperative regimens. *Am J Sports Med.* 2007;35:59-64.
58. Pajala A, Kangas J, Ohtonen P, Leppilahti J. Rerupture and deep infection following treatment of total Achilles tendon rupture. *J Bone Joint Surg Am.* 2002;84-A:2016-2021.
59. Leppilahti J, Forsman K, Puranen J, Orava S. Outcome and prognostic factors of achilles rupture repair using a new scoring method. *Clin Orthop Relat Res.* 1998;152-161.
60. September AV, Schweltnus MP, Collins M. Tendon and ligament injuries: the genetic component. *Br J Sports Med.* 2007;41:241-246.
61. van der Linden PD, Sturkenboom MC, Herings RM, et al. Increased risk of achilles tendon rupture with quinolone antibacterial use, especially in elderly patients taking oral corticosteroids. *Arch Intern Med.* 2003;163:1801-1807.
62. Jozsa L, Kvist M, Balint BJ, et al. The role of recreational sport activity in Achilles tendon rupture. A clinical, pathoanatomical, and sociological study of 292 cases. *Am J Sports Med.* 1989;17:338-343.
63. van der Linden PD, Sturkenboom MC, Herings RM, et al. Fluoroquinolones and risk of Achilles tendon disorders: case-control study. *Bmj.* 2002;324:1306-1307.
64. Jozsa L, Balint JB, Kannus P, et al. Distribution of blood groups in patients with tendon rupture. An analysis of 832 cases. *J Bone Joint Surg Br.* 1989;71:272-274.
65. Maffulli N, Reaper JA, Waterston SW, Ahya T. ABO blood groups and achilles tendon rupture in the Grampian Region of Scotland. *Clin J Sport Med.* 2000;10:269-271.
66. Corps AN, Harrall RL, Curry VA, et al. Ciprofloxacin enhances the stimulation of matrix metalloproteinase 3 expression by interleukin-1beta in human tendon-derived cells. A potential mechanism of fluoroquinolone-induced tendinopathy. *Arthritis Rheum.* 2002;46:3034-3040.
67. Corps AN, Harrall RL, Curry VA, et al. Contrasting effects of fluoroquinolone antibiotics on the expression of the collagenases, matrix metalloproteinases (MMP)-1 and -13, in human tendon-derived cells. *Rheumatology (Oxford).* 2005;44:1514-1517.
68. Reviglio VE, Hakim MA, Song JK, O'Brien TP. Effect of topical fluoroquinolones on the expression of matrix metalloproteinases in the cornea. *BMC Ophthalmol.* 2003;3:10.
69. Oshiro W, Lou J, Xing X, et al. Flexor tendon healing in the rat: a histologic and gene expression study. *J Hand Surg [Am].* 2003;28:814-823.
70. Soo C, Shaw WW, Zhang X, et al. Differential expression of matrix metalloproteinases and their tissue-derived inhibitors in cutaneous wound repair. *Plast Reconstr Surg.* 2000;105:638-647.
71. Hatanaka H, Zhang J, Manske PR. An in vivo study of locking and grasping techniques using a passive mobilization protocol in experimental animals. *J Hand Surg [Am].* 2000;25:260-269.
72. Wada A, Kubota H, Miyanishi K, et al. Comparison of postoperative early active mobilization and immobilization in vivo utilising a four-strand flexor tendon repair. *J Hand Surg [Br].* 2001;26:301-306.
73. Yildirim Y, Kara H, Cabukoglu C, Esemeli T. Suture holding capacity of the Achilles tendon during the healing period: an in vivo experimental study in rabbits. *Foot Ankle Int.* 2006;27:121-124.
74. McDowell CL, Marqueen TJ, Yager D, et al. Characterization of the tensile properties and histologic/biochemical changes in normal chicken tendon at the site of suture insertion. *J Hand Surg [Am].* 2002;27:605-614.
75. Harris SB, Harris D, Foster AJ, Elliot D. The aetiology of acute rupture of flexor tendon repairs in zones 1 and 2 of the fingers during early mobilization. *J Hand Surg [Br].* 1999;24:275-280.
76. Lynch RM. Achilles tendon rupture: surgical versus non-surgical treatment. *Accid Emerg Nurs.* 2004;12:149-158.
77. Ejeskar A, Irtam L. Elongation in profundus tendon repair. A clinical and radiological study. *Scand J Plast Reconstr Surg.* 1981;15:61-68.
78. Gelberman RH, Boyer MI, Brodt MD, et al. The effect of gap formation at the repair site on the strength and excursion of intrasynovial flexor tendons. An experimental study on the early stages of tendon-healing in dogs. *J Bone Joint Surg Am.* 1999;81:975-982.
79. Seradge H. Elongation of the repair configuration following flexor tendon repair. *J Hand Surg [Am].* 1983;8:182-185.
80. Chambers WM, Mortensen NJ. Postoperative leakage and abscess formation after colorectal surgery. *Best Pract Res Clin Gastroenterol.* 2004;18:865-880.
81. Hendriks T, Mastboom WJ. Healing of experimental intestinal anastomoses. Parameters for repair. *Dis Colon Rectum.* 1990;33:891-901.

82. Agren MS, Jorgensen LN, Delaisse JM. Matrix metalloproteinases and colon anastomosis repair: a new indication for pharmacological inhibition? *Mini Rev Med Chem.* 2004;4:769-778.
83. de Hingh IH, de Man BM, Lomme RM, et al. Colonic anastomotic strength and matrix metalloproteinase activity in an experimental model of bacterial peritonitis. *Br J Surg.* 2003;90:981-988.
84. Agren MS, Andersen TL, Mirastschijski U, et al. Action of matrix metalloproteinases at restricted sites in colon anastomosis repair: an immunohistochemical and biochemical study. *Surgery.* 2006;140:72-82.
85. Savage FJ, Lacombe DL, Boulos PB, Hembry RM. Role of matrix metalloproteinases in healing of colonic anastomosis. *Dis Colon Rectum.* 1997;40:962-970.
86. Stumpf M, Klinge U, Wilms A, et al. Changes of the extracellular matrix as a risk factor for anastomotic leakage after large bowel surgery. *Surgery.* 2005;137:229-234.
87. Syk I, Agren MS, Adawi D, Jeppsson B. Inhibition of matrix metalloproteinases enhances breaking strength of colonic anastomoses in an experimental model. *Br J Surg.* 2001;88:228-234.
88. Siemonsma MA, de Hingh IH, de Man BM, et al. Doxycycline improves wound strength after intestinal anastomosis in the rat. *Surgery.* 2003;133:268-276.
89. Sheridan WG, Shandall AA, Alexander-Williams J, et al. A multicenter trial of the use of the proteolytic enzyme inhibitor aprotinin in colorectal surgery. *Dis Colon Rectum.* 1989;32:505-508.
90. Watelet JB, Claeys C, Van Cauwenberge P, Bachert C. Predictive and monitoring value of matrix metalloproteinase-9 for healing quality after sinus surgery. *Wound Repair Regen.* 2004;12:412-418.
91. Agren MS, Jorgensen LN, Andersen M, et al. Matrix metalloproteinase 9 level predicts optimal collagen deposition during early wound repair in humans. *Br J Surg.* 1998;85:68-71.
92. Henle P, Zimmermann G, Weiss S. Matrix metalloproteinases and failed fracture healing. *Bone.* 2005;37:791-798.
93. Malik MH, Jury F, Bayat A, et al. Genetic susceptibility to total hip arthroplasty failure: a preliminary study on the influence of matrix metalloproteinase 1, interleukin 6 polymorphisms and vitamin D receptor. *Ann Rheum Dis.* 2007;66:1116-1120.
94. Lee CZ, Xu B, Hashimoto T, et al. Doxycycline suppresses cerebral matrix metalloproteinase-9 and angiogenesis induced by focal hyperstimulation of vascular endothelial growth factor in a mouse model. *Stroke.* 2004;35:1715-1719.
95. Tamargo RJ, Bok RA, Brem H. Angiogenesis inhibition by minocycline. *Cancer Res.* 1991;51:672-675.
96. Sapadin AN, Fleischmajer R. Tetracyclines: nonantibiotic properties and their clinical implications. *J Am Acad Dermatol.* 2006;54:258-265.
97. Amin AR, Attur MG, Thakker GD, et al. A novel mechanism of action of tetracyclines: effects on nitric oxide synthases. *Proc Natl Acad Sci U S A.* 1996;93:14014-14019.
98. Kloppenburg M, Brinkman BM, de Rooij-Dijk HH, et al. The tetracycline derivative minocycline differentially affects cytokine production by monocytes and T lymphocytes. *Antimicrob Agents Chemother.* 1996;40:934-940.
99. Golub LM, Lee HM, Ryan ME, et al. Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. *Adv Dent Res.* 1998;12:12-26.
100. Yrjanheikki J, Tikka T, Keinanen R, et al. A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc Natl Acad Sci U S A.* 1999;96:13496-13500.
101. Peterson JT. Matrix metalloproteinase inhibitor development and the remodeling of drug discovery. *Heart Fail Rev.* 2004;9:63-79.
102. O'Dell JR, Elliott JR, Mallek JA, et al. Treatment of early seropositive rheumatoid arthritis: doxycycline plus methotrexate versus methotrexate alone. *Arthritis Rheum.* 2006;54:621-627.
103. Emingil G, Atilla G, Sorsa T, et al. The effect of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid matrix metalloproteinase-8 levels in chronic periodontitis. *J Periodontol.* 2004;75:106-115.
104. Walker C, Preshaw PM, Novak J, et al. Long-term treatment with sub-antimicrobial dose doxycycline has no antibacterial effect on intestinal flora. *J Clin Periodontol.* 2005;32:1163-1169.
105. Hu J, Van den Steen PE, Sang QX, Opdenakker G. Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. *Nat Rev Drug Discov.* 2007;6:480-498.
106. Teronen O, Heikkilä P, Kontinen YT, et al. MMP inhibition and downregulation by bisphosphonates. *Ann N Y Acad Sci.* 1999;878:453-465.
107. Heikkilä P, Teronen O, Moilanen M, et al. Bisphosphonates inhibit stromelysin-1 (MMP-3), matrix metalloelastase (MMP-12), collagenase-3 (MMP-13) and enamelysin (MMP-20), but not urokinase-type plasminogen activator, and diminish invasion and migration of human malignant and endothelial cell lines. *Anticancer Drugs.* 2002;13:245-254.

108. Arnoczky SP, Lavagnino M, Egerbacher M, et al. Matrix metalloproteinase inhibitors prevent a decrease in the mechanical properties of stress-deprived tendons: an in vitro experimental study. *Am J Sports Med.* 2007;35:763-769.
109. Bastos LF, Merlo LA, Rocha LT, Coelho MM. Characterization of the antinociceptive and anti-inflammatory activities of doxycycline and minocycline in different experimental models. *Eur J Pharmacol.* 2007.
110. Yong VW, Giuliani F, Xue M, et al. Experimental models of neuroprotection relevant to multiple sclerosis. *Neurology.* 2007;68:S32-37; discussion S43-54.
111. Xu L, Fagan SC, Waller JL, et al. Low dose intravenous minocycline is neuroprotective after middle cerebral artery occlusion-reperfusion in rats. *BMC Neurol.* 2004;4:7.
112. Henry SL, Concannon MJ, Kaplan PA, Diaz-Arias AA. The inhibitory effect of minocycline on hypertrophic scarring. *Plast Reconstr Surg.* 2007;120:80-88; discussion 89-90.
113. Mosorin M, Juvonen J, Biancari F, et al. Use of doxycycline to decrease the growth rate of abdominal aortic aneurysms: a randomized, double-blind, placebo-controlled pilot study. *J Vasc Surg.* 2001;34:606-610.
114. O'Dell JR, Blakely KW, Mallek JA, et al. Treatment of early seropositive rheumatoid arthritis: a two-year, double-blind comparison of minocycline and hydroxychloroquine. *Arthritis Rheum.* 2001;44:2235-2241.
115. Zabad RK, Metz LM, Todoruk TR, et al. The clinical response to minocycline in multiple sclerosis is accompanied by beneficial immune changes: a pilot study. *Mult Scler.* 2007;13:517-526.
116. Steinberg J, Halter J, Schiller H, et al. Chemically modified tetracycline prevents the development of septic shock and acute respiratory distress syndrome in a clinically applicable porcine model. *Shock.* 2005;24:348-356.
117. Dezube BJ, Krown SE, Lee JY, et al. Randomized phase II trial of matrix metalloproteinase inhibitor COL-3 in AIDS-related Kaposi's sarcoma: an AIDS Malignancy Consortium Study. *J Clin Oncol.* 2006;24:1389-1394.
118. Witte MB, Thornton FJ, Kiyama T, et al. Metalloproteinase inhibitors and wound healing: a novel enhancer of wound strength. *Surgery.* 1998;124:464-470.
119. Leib SL, Clements JM, Lindberg RL, et al. Inhibition of matrix metalloproteinases and tumour necrosis factor alpha converting enzyme as adjuvant therapy in pneumococcal meningitis. *Brain.* 2001;124:1734-1742.
120. Schepull T, Kvist J, Andersson C, Aspenberg P. Mechanical properties during healing of Achilles tendon ruptures to predict final outcome. A pilot Roentgen stereophotogrammetric analysis in 10 patients. *BMC Musculoskelet Disord.* 2007;8:116.
121. Tengvall P, Jansson, E, Askendel, A, Thomsen, P, Gretzer, C. Preparation of multilayer plasma protein films on silicon by EDC/NHS coupling chemistry. *Colloids and Surfaces B Biointerfaces.* 2003;28:261-272.
122. McCrackin FL. A FORTAN program for the analysis of ellipsometer measurements. *NBC technical note.* Washington DC, USA; 1969:479.
123. De Feijter JA, Benjamins, J, Veer, FA. Ellipsometry as a tool to study the adsorption behavior of synthetic and biopolymers at the air-water interface. *Biopolymers.* 1978;17:1759 - 1772.
124. Benesch J, Askendal, A, Tengvall, P. Quantification of adsorbed human serum albumin at solid interfaces: a comparison between radioimmunoassay (RIA) and simple null ellipsometry. *Colloids and Surfaces B Biointerfaces.* 2000;18:71-81.
125. Stenberg M, Nygren, H. The use of the isoscope ellipsometer in the study of adsorbed proteins and biospecific binding reactions. *J de Physique.* 1983;C10:83-86.
126. Klassen M, Edberg, SC. Measurement of antibiotics in human body fluids: Techniques and significance. In: Lorian V, ed. *Antibiotics in Laboratory Medicine.* 4th ed. New York: Williams & Wilkins; 1996.
127. Thrailkill KM, Moreau CS, Cockrell G, et al. Physiological matrix metalloproteinase concentrations in serum during childhood and adolescence, using Luminex Multiplex technology. *Clin Chem Lab Med.* 2005;43:1392-1399.
128. Lo IK, Marchuk LL, Hollinshead R, et al. Matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase mRNA levels are specifically altered in torn rotator cuff tendons. *Am J Sports Med.* 2004;32:1223-1229.
129. Prall AK, Longo GM, Mayhan WG, et al. Doxycycline in patients with abdominal aortic aneurysms and in mice: comparison of serum levels and effect on aneurysm growth in mice. *J Vasc Surg.* 2002;35:923-929.
130. Sorsa T, Tjaderhane L, Konttinen YT, et al. Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Ann Med.* 2006;38:306-321.

131. Gnarpe H, Dornbusch K, Hagg O. Doxycycline concentration levels in bone, soft tissue and serum after intravenous infusion of doxycycline. A clinical study. *Scand J Infect Dis Suppl.* 1976;54-57.
132. Hojer H, Wetterfors J. Systemic prophylaxis with doxycycline in surgery of the colon and rectum. *Ann Surg.* 1978;187:362-368.
133. Virchenko O, Skoglund B, Aspenberg P. Parecoxib impairs early tendon repair but improves later remodeling. *Am J Sports Med.* 2004;32:1743-1747.
134. Demirag B, Sarisozen B, Ozer O, et al. Enhancement of tendon-bone healing of anterior cruciate ligament grafts by blockage of matrix metalloproteinases. *J Bone Joint Surg Am.* 2005;87:2401-2410.
135. Zhao C, Amadio PC, Momose T, et al. Effect of synergistic wrist motion on adhesion formation after repair of partial flexor digitorum profundus tendon lacerations in a canine model in vivo. *J Bone Joint Surg Am.* 2002;84-A:78-84.
136. Tengvall P, Skoglund B, Askendal A, Aspenberg P. Surface immobilized bisphosphonate improves stainless-steel screw fixation in rats. *Biomaterials.* 2004;25:2133-2138.
137. Wermelin K, Tengvall P, Aspenberg P. Surface-bound bisphosphonates enhance screw fixation in rats-increasing effect up to 8 weeks after insertion. *Acta Orthop.* 2007;78:385-392.
138. Hilding M, Aspenberg P. Local perioperative treatment with a bisphosphonate improves the fixation of total knee prostheses: A randomized, double-blind radiostereometric study of 50 patients. *Acta Orthop.* 2007;78:795-799.
139. Junge K, Rosch R, Bialasinski L, et al. Persistent extracellular matrix remodelling at the interface to polymers used for hernia repair. *Eur Surg Res.* 2003;35:497-504.
140. Mansson P, Zhang XW, Jeppsson B, Thorlacius H. Anastomotic healing in the rat colon: comparison between a radiological method, breaking strength and bursting pressure. *Int J Colorectal Dis.* 2002;17:420-425.
141. Ikeuchi D, Onodera H, Aung T, et al. Correlation of tensile strength with bursting pressure in the evaluation of intestinal anastomosis. *Dig Surg.* 1999;16:478-485.
142. Hutchinson JW, Tierney GM, Parsons SL, Davis TR. Dupuytren's disease and frozen shoulder induced by treatment with a matrix metalloproteinase inhibitor. *J Bone Joint Surg Br.* 1998;80:907-908.
143. Krzeski P, Buckland-Wright C, Balint G, et al. Development of musculoskeletal toxicity without clear benefit after administration of PG-116800, a matrix metalloproteinase inhibitor, to patients with knee osteoarthritis: a randomized, 12-month, double-blind, placebo-controlled study. *Arthritis Res Ther.* 2007;9:R109.
144. Tekin A, Yol S, Yilmaz H, et al. Effects of Platelet Rich Plasma on Colonic Anastomosis. *J Surg Res.* 2007.
145. Yarimkaya A, Apaydin B, Unal E, et al. Effects of recombinant human growth hormone and nandrolone phenylpropionate on the healing of ischemic colon anastomosis in rats. *Dis Colon Rectum.* 2003;46:1690-1697.
146. Zacharakis E, Demetriades H, Kanellos D, et al. Contribution of insulin-like growth factor I to the healing of colonic anastomoses in rats. *J Invest Surg.* 2007;20:9-14.
147. Kiyama T, Onda M, Tokunaga A, et al. Effect of matrix metalloproteinase inhibition on colonic anastomotic healing in rats. *J Gastrointest Surg.* 2001;5:303-311.
148. Balcom JH, Keck T, Warshaw AL, et al. Perioperative matrix metalloproteinase inhibition therapy does not impair wound or anastomotic healing. *J Gastrointest Surg.* 2002;6:488-495.
149. de Hingh IH, Siemonsma MA, de Man BM, et al. The matrix metalloproteinase inhibitor BB-94 improves the strength of intestinal anastomoses in the rat. *Int J Colorectal Dis.* 2002;17:348-354.
150. Frederiks WM, Mook OR. Metabolic mapping of proteinase activity with emphasis on in situ zymography of gelatinases: review and protocols. *J Histochem Cytochem.* 2004;52:711-722.
151. Sukhova GK, Schonbeck U, Rabkin E, et al. Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atheromatous plaques. *Circulation.* 1999;99:2503-2509.
152. Josefsson G, Lindberg L, Wiklander B. Systemic antibiotics and gentamicin-containing bone cement in the prophylaxis of postoperative infections in total hip arthroplasty. *Clin Orthop Relat Res.* 1981:194-200.
153. Vintar N, Rawal N, Veselko M. Intraarticular patient-controlled regional anesthesia after arthroscopically assisted anterior cruciate ligament reconstruction: ropivacaine/morphine/ketorolac versus ropivacaine/morphine. *Anesth Analg.* 2005;101:573-578, table of contents.
154. Dines JS, Weber L, Razzano P, et al. The effect of growth differentiation factor-5-coated sutures on tendon repair in a rat model. *J Shoulder Elbow Surg.* 2007;16:S215-221.
155. Hamada Y, Katoh S, Hibino N, et al. Effects of monofilament nylon coated with basic fibroblast growth factor on endogenous intrasynovial flexor tendon healing. *J Hand Surg [Am].* 2006;31:530-540.
156. Rickert M, Jung M, Adiyaman M, et al. A growth and differentiation factor-5 (GDF-5)-coated suture stimulates tendon healing in an Achilles tendon model in rats. *Growth Factors.* 2001;19:115-126.

157. Mazzocca AD, McCarthy MB, Arciero C, et al. Tendon and bone responses to a collagen-coated suture material. *J Shoulder Elbow Surg.* 2007;16:S222-230.
158. Rohrich RJ, Trott SA, Love M, et al. Mersilene suture as a vehicle for delivery of growth factors in tendon repair. *Plast Reconstr Surg.* 1999;104:1713-1717.
159. Jung K. Preanalytical biases in measurement of matrix metalloproteinases and their tissue inhibitors in peripheral blood. *J Rheumatol.* 2007;34:890-892.
160. Thraikill K, Cockrell G, Simpson P, et al. Physiological matrix metalloproteinase (MMP) concentrations: comparison of serum and plasma specimens. *Clin Chem Lab Med.* 2006;44:503-504.
161. Tuomainen AM, Nyyssonen K, Tervahartiala T, et al. Matrix metalloproteinase-8 and tissue inhibitor of metalloproteinase-1 in serum do not reflect the analytes circulating in blood. *Arterioscler Thromb Vasc Biol.* 2008;28:e17.
162. Bender R, Lange S. Adjusting for multiple testing--when and how? *J Clin Epidemiol.* 2001;54:343-349.
163. Perneger TV. What's wrong with Bonferroni adjustments. *Bmj.* 1998;316:1236-1238.
164. Shalabi A, Svensson L, Kristoffersen-Wiberg M, et al. Tendon injury and repair after core biopsies in chronic Achilles tendinosis evaluated by serial magnetic resonance imaging. *Br J Sports Med.* 2004;38:606-612.
165. Liden M, Movin T, Ejerhed L, et al. A Histological and Ultrastructural Evaluation of the Patellar Tendon 10 Years After Reharvesting Its Central Third. *Am J Sports Med.* 2008.
166. Svensson M, Kartus J, Christensen LR, et al. A long-term serial histological evaluation of the patellar tendon in humans after harvesting its central third. *Knee Surg Sports Traumatol Arthrosc.* 2005;13:398-404.
167. Kartus J, Movin T, Papadogiannakis N, et al. A radiographic and histologic evaluation of the patellar tendon after harvesting its central third. *Am J Sports Med.* 2000;28:218-226.
168. Santos MC, Campos MI, Souza AP, et al. Analysis of MMP-1 and MMP-9 promoter polymorphisms in early osseointegrated implant failure. *Int J Oral Maxillofac Implants.* 2004;19:38-43.
169. Zheng H, Si Z, Kasperk R, et al. Recurrent inguinal hernia: disease of the collagen matrix? *World J Surg.* 2002;26:401-408.
170. Rosch R, Klinge U, Si Z, et al. A role for the collagen I/III and MMP-1/-13 genes in primary inguinal hernia? *BMC Med Genet.* 2002;3:2.
171. Bellon JM, Bajo A, Ga-Honduvilla N, et al. Fibroblasts from the transversalis fascia of young patients with direct inguinal hernias show constitutive MMP-2 overexpression. *Ann Surg.* 2001;233:287-291.
172. Pleumeekers HJ, De Gruijl A, Hofman A, et al. Prevalence of aortic aneurysm in men with a history of inguinal hernia repair. *Br J Surg.* 1999;86:1155-1158.
173. Lohmander LS, Brandt KD, Mazzuca SA, et al. Use of the plasma stromelysin (matrix metalloproteinase 3) concentration to predict joint space narrowing in knee osteoarthritis. *Arthritis Rheum.* 2005;52:3160-3167.
174. Zimmermann G, Henle P, Kusswetter M, et al. TGF-beta1 as a marker of delayed fracture healing. *Bone.* 2005;36:779-785.