

# Ciprofloxacin Up-Regulates Tendon Cells to Express Matrix Metalloproteinase-2 with Degradation of Type I Collagen

Wen-Chung Tsai,<sup>1</sup> Chih-Chin Hsu,<sup>2</sup> Carl P.C. Chen,<sup>1</sup> Hsiang-Ning Chang,<sup>1</sup> Alice M.K. Wong,<sup>1</sup> Miao-Sui Lin,<sup>1</sup> Jong-Hwei S. Pang<sup>3</sup>

<sup>1</sup>Department of Physical Medicine and Rehabilitation, Chang Gung Memorial Hospital at Linkou, College of Medicine, Chang Gung University, Linkou, Taiwan, <sup>2</sup>Department of Physical Medicine and Rehabilitation, Chang Gung Memorial Hospital at Keelung, College of Medicine, Chang Gung University, Taiwan, <sup>3</sup>Graduate Institute of Clinical Medical Sciences, Chang Gung University, Taiwan

Received 22 January 2010; accepted 19 May 2010

Published online 2 July 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.21196

**ABSTRACT:** Ciprofloxacin-induced tendinopathy and tendon rupture have been previously described, principally affecting the Achilles tendon. This study was designed to investigate the effect of ciprofloxacin on expressions of matrix metalloproteinases (MMP)-2 and -9, tissue inhibitors of metalloproteinase (TIMP)-1 and -2 as well as type I collagen in tendon cells. Tendon cells intrinsic to rat Achilles tendon were treated with ciprofloxacin and then underwent MTT (tetrazolium) assay. Real-time reverse-transcription polymerase chain reaction (RT-PCR) and Western blot analysis were used, respectively, to evaluate the gene and protein expressions of type I collagen, and MMP-2. Gelatin zymography was used to evaluate the enzymatic activities of MMP-2 and -9. Reverse zymography was used to evaluate TIMP-1 and -2. Immunohistochemical staining for MMP-2 in ciprofloxacin-treated tendon explants was performed. Collagen degradation was evaluated by incubation of conditioned medium with collagen. The results revealed that ciprofloxacin up-regulated the expression of MMP-2 in tendon cells at the mRNA and protein levels. Immunohistochemistry also confirmed the increased expressions of MMP-2 in ciprofloxacin-treated tendon explants. The enzymatic activity of MMP-2 was up-regulated whereas that of MMP-9, TIMP-1 or TIMP-2 was unchanged. The amount of secreted type I collagen in the conditioned medium decreased and type I collagen was degraded after ciprofloxacin treatment. In conclusion, ciprofloxacin up-regulates the expressions of MMP-2 in tendon cells and thus degraded type I collagen. These findings suggest a possible mechanism of ciprofloxacin-associated tendinopathy. © 2010 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. *J Orthop Res* 29: 67–73, 2011

**Keywords:** tendon; ciprofloxacin; matrix metalloproteinase; collagen

The fluoroquinolone class of antibiotics (e.g., ciprofloxacin, levofloxacin, moxifloxacin) has been used to treat a wide range of infections. It was reported in the literature that quinolone-induced tendinopathy or even tendon rupture principally affected the Achilles tendon.<sup>1,2</sup> However, the mechanisms by which ciprofloxacin predisposes tendinopathy or even tendon rupture remain to be investigated.

In animal studies with fluoroquinolone-treated rats, disorganization of the extracellular matrix (ECM), inflammation of the paratenon and degenerative changes in tendon cells have been demonstrated.<sup>3,4</sup> Besides, fluoroquinolone class of antibiotics has been documented to exert a number of effects on various cell types *in vitro*, including reduced expression of some ECM proteins,<sup>5,6</sup> decreased mitochondrial activity,<sup>6</sup> enhanced matrix metalloproteinase (MMP) expression,<sup>5,7</sup> noncytotoxic inhibition of tendon cell proliferation<sup>5,8</sup> and inhibition of tendon cell migration.<sup>9</sup> Further matrix degradation or repeated micro-trauma to a tendinopathic tendon might inevitably lead to a tendon rupture.

The basic constituent of a tendon is collagen, which accounts for 70% of the dry weight of a tendon.<sup>10</sup> Approximately 90% of collagen in normal tendons is type I and less than 10% is type III collagen.<sup>11</sup> Type I collagen is organized into fibrils grouped in parallel to form

organized bundles while type III collagen is almost completely confined to the endotendineum which surrounds the bundles.<sup>12</sup> Tendon cells (fibroblasts), which are its basic cellular component of a tendon, are the source of collagen production, protein mediators of repair, and matrix proteoglycans.<sup>10</sup> It appears that the physiologic response of tendon cells to trauma induces production of both types I and III collagen.<sup>13</sup> The biomechanical properties of a tendon are primarily a feature of the ECM (mainly collagen), which is in a state of dynamic equilibrium between synthesis and degradation.<sup>14</sup>

Gelatinase such as MMP-2 and MMP-9 are MMPs with collagenolytic activity.<sup>15,16</sup> The activity of MMP is inhibited by tissue inhibitors of MMPs (TIMPs)<sup>17,18</sup> and the balance in MMPs and TIMPs regulated tendon remodeling. The expression of MMP-2 was up-regulated in Achilles tendinopathy and MMP-9 expression was also up-regulated in the ruptured area of Achilles tendon.<sup>19,20</sup> It is concerned that the potential combination of increased local matrix-degrading activity by enhanced MMP expression and/or decreased ECM production in tendon cells after ciprofloxacin treatment might induce the occurrence of tendinopathy or tendon rupture. The aim of this study is to investigate the effect of ciprofloxacin on expressions of type I collagen, MMP-2, MMP-9, TIMP-1, and TIMP-2 of tendon cells.

## METHODS

All procedures were approved by the Institutional Animal Care and Use Committee.

Correspondence to: Jong-Hwei S. Pang (T: 886-3-2118800, ext. 3482; F: 886-3-3280170; E-mail: jonghwei@mail.cgu.edu.tw)  
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### Primary Culture of Rat Achilles Tendon Cells

Tendon cells were obtained from Sprague–Dawley rats (200–250 g) as previously described.<sup>21</sup> Samples from passages 2 to 4, with proper growth rate and normal fibroblast shape, were used. All the following experiments were performed at least in triplicate.

### MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-Diphenyltetrazolium Bromide) Assay

Tendon cells were left untreated or treated with 5, 10, 20, and 50 µg/mL ciprofloxacin. Cell survival was determined by MTT assay 24 h after treatment. MTT (50 µg/mL) was added and incubated at 37°C for 1 h. Then, the MTT solution was discarded and 1 mL DMSO was added to dissolve formazan crystals. Optical density at 570 nm (OD 570 nm) in aliquots was read using a spectrophotometer (Nano Drop 1000, Thermo Scientific, Wilmington, DE). Percentages of OD value for ciprofloxacin treated cells relative to the control were calculated.

### Quantitative Real-Time RT-PCR Analysis

Total RNA was extracted from tendon cells using solution D (1 mL solution D/10<sup>7</sup> cells). Subsequently, total RNA was extracted with phenol and chloroform/isoamyl alcohol (49:1) to remove proteins and genomic DNAs. Complementary (c) DNA was synthesized using 1 mg total RNA in a 20 mL volume RT reaction mix containing 0.5 mg of random primers, 0.8 mM dNTP, 0.1 M DTT, and 1 L first strand buffer. Quantitative real-time RT-PCR was performed using an SYBR Green and MxPro-Mx3000P QPCR machine (Stratagene, NeoMarkers, Fremont, CA). Aliquots (20 ng) of cDNA were used for each quantitative PCR, and each reaction was run in triplicate. The following primers were used: GAPDH: 5'-TTCATTGACCTCAACTACAT-3' (forward) and 5'-GAGGGGCCATCCACAGTCTT-3' (backward), type I collagen: 5'-TGGAGACAGGTCAGACCTG-3' (forward) and 5'-TATTCGATGACTGTCTTGCC-3' (reverse), as well as MMP-2: 5'-GGAAGCATCAAATCGGACTG-3' (forward) and 5'-GGCGGGGAGAAAGTAGCA-3' (reverse). Relative gene expressions between experimental groups were determined using MxPro software (Stratagene) and GAPDH was used as an internal control. All real-time RT-PCRs were performed in triplicate, and changes in gene expressions were reported as multiples of increases relative to the untreated controls.

### Western Blot Analysis

The levels of MMP-2 in the conditioned medium were analyzed by Western blot analysis. The methods were described as previous study.<sup>9</sup> Primary antibodies such as mouse monoclonal antibody against tubulin, type I collagen, and MMP-2 were used. The semi-quantitative measurement of the band density was calculated by using 1D Digital Analysis Software (Kodak Digital Science™, Eastman Kodak, Rochester, NY). The band densities were normalized to relative cell number with the results being band density divided by the percentage of cell number from the results of corresponding MTT assay. Normalized data were expressed as 100% in control group.

### Gelatin Zymography

MMP-2 and -9 in conditioned medium were detected by gelatin zymography. It was performed under nonreducing conditions using a 7.5 % SDS–polyacrylamide gel containing 2 mg/mL gelatin (Mini-PROTEAN II system, Bio-Rad Laboratories Ltd, Hemphstead, UK). Gels were washed in 2.5% Triton X-100 to

remove SDS and allow renaturation of MMPs, then transferred to 50 mM Tris (pH 7.5), 5 mM CaCl<sub>2</sub>, 1 mM ZnCl<sub>2</sub> and incubated at 37°C for 18 h. After staining with Coomassie brilliant blue R250 (Bio-Rad Laboratories, Hercules, CA), pro-MMPs and active MMPs result in white lysis bands, due to gelatin degradation.

### Reverse Zymography

Reverse zymography was performed similarly as zymography with the exception that conditioned medium which contained activities of MMP-2 and -9, was included in the gel mix except gelatin. All washes and incubations procedures were the same as for zymography. TIMP-1 and -2, which inhibited gelatin digestion by MMP-2 and -9, appeared as dark bands on a white background.

### Ex Vivo Experimental Design and Immunohistochemistry

Achilles tendon (six tendons from three rats) was chopped into two pieces and put separately into two dishes. Culture medium made of Dulbecco's modified Eagle's medium (DMEM; HyClone, Logan, UT), with 10% FBS (Cansera, Rexdale, ON, Canada), 100 U/mL penicillin, and 100 mg/mL streptomycin in the presence or absence of ciprofloxacin was added for 24 h. The paraffin-embedded tissue sections were stained by the standard immunoperoxidase staining procedure. In brief, the paraffin embedded tendon blocks were cut into 5 µm sections, de-paraffinized, washed and then sequentially blocked for endogenous peroxidase activity with 3% H<sub>2</sub>O<sub>2</sub>, and nonspecific antibody binding sites with 1% BSA and 1% goat serum. After three washings in PBS, the tissue sections were incubated for 1 h with mouse monoclonal antibodies against MMP-2 (Neo-Marks, Fremont, CA) diluted in blocking solution. Negative control was performed following the same procedures except incubation with primary antibody. The signal was detected with DAKO labeled streptavidin–biotin system and color development was performed by incubation with diaminobenzidine substrate-chromogen (DAKO, Via Real, Carpinteria, CA) for 5 min.

### Collagen Degradation

The condition media of tenocytes treated with or without various concentrations of ciprofloxacin (5, 10, 20, and 50 µg/mL) were collected and then mixed with collagen (0.5 mg/mL) extracted from tendon (1:1). After incubation in 37°C for 24 h, the reaction products were revealed by nondenaturing gel electrophoresis. The collagen degradation, as demonstrated by the decreased amount (band density) of collagen, was observed after the gel was stained with Coomassie blue.

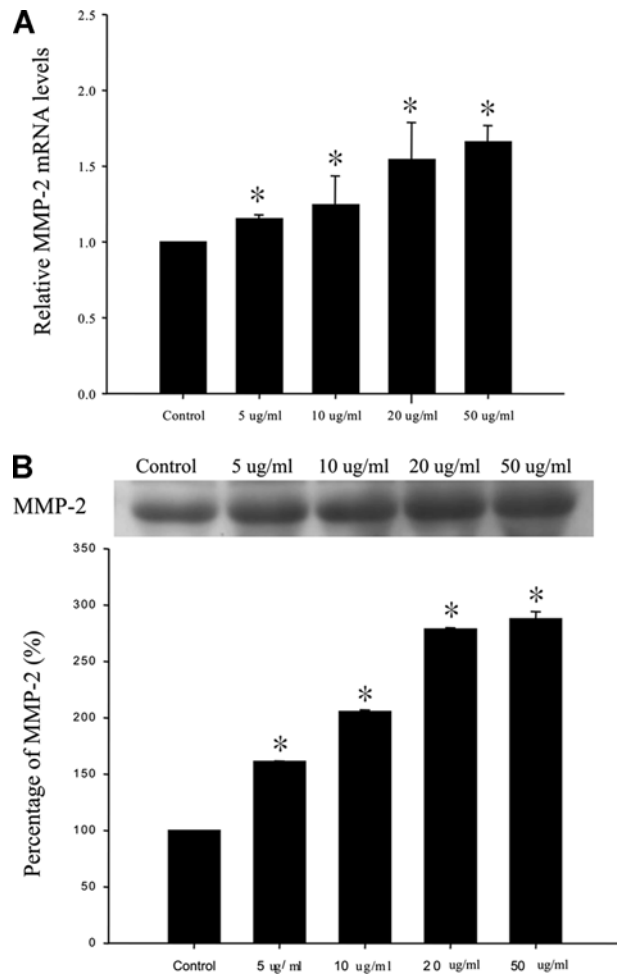
### Statistical Analysis

All nonparametric data obtained by MTT assay and densitometric analysis were expressed as the mean ± SEM. Ciprofloxacin-treated and control cells were compared using the Kruskal–Wallis test. The Mann–Whitney test was used to determine the significance of differences. *p* values less than 0.05 were considered significant.

## RESULTS

### The Effect of Ciprofloxacin on Tendon Cell Viability

MTT data revealed that ciprofloxacin reduced relative OD 570 nm in a dose-dependent manner. The OD value relative to control group was 100.1 ± 1.6%, 90.8 ± 0.2%, 80.8 ± 4.4%, and 74.5 ± 2.0% for cultures treated with 5, 10, 20, and 50 µg/mL ciprofloxacin, respectively. These



**Figure 1.** The results of real-time RT-PCR (A) and densitometric analysis of the results of Western blot analysis normalized to cell number (MTT result) (B) (\* $p < 0.05$ ,  $n = 3$ ).

results indicated that ciprofloxacin decreased the cellularity of tendon cells ( $p < 0.05$ ).

#### The Effect of Ciprofloxacin on mRNA and Protein Expressions of MMP-2

The results of real-time RT-PCR revealed that gene expression of MMP-2 was up-regulated by ciprofloxacin dose-dependently (Fig. 1A). Densitometric analysis for the Western blot analysis for the amounts of secreted MMP-2 normalized to cell number (MTT results) revealed that the protein expression of MMP-2 (Fig. 1B) was also up-regulated by ciprofloxacin dose dependently. The difference between control and ciprofloxacin treated cells were statistically significant ( $p < 0.05$ ).

#### The Effect of Ciprofloxacin on Enzymatic Activities of MMP-2 and -9

Result of gelatin zymography that analyzed the activities of MMP-2 and MMP-9 was shown in Figure 2A. The major gelatinase activity in tendon cells was found to be MMP-2, but not MMP-9. The MMP-2 activities from different samples analyzed by densitometric method, after subtracting the background value, were normal-

ized to cell number (MTT results) and dose-dependent increase of MMP-2 activities was demonstrated after ciprofloxacin treatment (Fig. 2B). However, the MMP-9 activities remained unchanged (Fig. 2C).

#### The Effect of Ciprofloxacin on Enzymatic Activities of TIMP-1 and TIMP-2

Result of reverse zymography that analyzed the activities of TIMP-1 and -2 was shown in Figure 3A. The densitometric analysis of TIMP-1 and -2 activities after normalization to cell number remained unchanged after ciprofloxacin treatment (Fig. 3B and C).

#### The Ex Vivo Effect of Ciprofloxacin on Expression of MMP-2 in Tendon Explants

Immunohistochemical staining for MMP-2 in tendon explants was further performed to validate whether the same results could be found ex vivo (Fig. 4). Tendon explants treated with 50 µg/mL ciprofloxacin (Fig. 4D) revealed more significant brown staining within the cytoplasm of tendon cells as compared to the control explants (Fig. 4C), indicating increased expression of MMP-2 in tendon treated with ciprofloxacin.

#### The Effect of Ciprofloxacin on Type I Collagen Expression

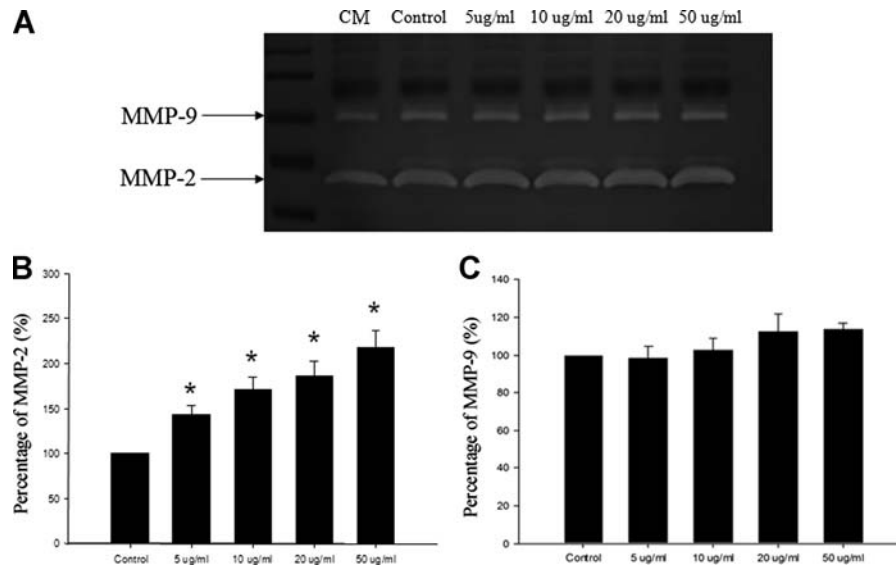
As shown in Figure 5A and B, both the mRNA and cytosolic protein expressions of type I collagen as reflected by real-time RT-PCR and Western blot analysis, respectively, remained unchanged in tendon cells after treatment with ciprofloxacin for 24 h. In contrast, the amount of secreted type I collagen in conditioned medium was dose-dependently decreased by ciprofloxacin (Fig. 5A and C), which was correlated well with the increase of MMP-2 activities in the conditioned medium.

#### Collagen Degradation

The result showed that more type I collagen was degraded by the conditioned medium from tenocytes treated with ciprofloxacin (Fig. 6). The dose-dependency was expected to be more significant when the band intensity was normalized by the cellularity of each sample.

#### DISCUSSION

There are evidences indicating that the increased expressions of certain MMPs are associated with tendinopathy or tendon rupture. Immunohistochemistry studies have shown MMP-1 expressed at the tear edge in ruptured supraspinatus tendons<sup>22</sup> and in patellar tendinosis.<sup>23</sup> Meanwhile, there is an increased amount of denatured collagen and MMP-1 in ruptured supraspinatus tendon which implied the increase in MMP-1 activity and degradation of the collagen fibril network might be a potential cause of a tendon rupture.<sup>24</sup> The up-regulation of MMP-13 at both the mRNA and protein levels was demonstrated in patients with complete tears of rotator cuff tendons.<sup>25</sup> In accordance with this study, an animal model revealed that



**Figure 2.** (A) Zymography of conditioned medium (CM) revealed that the enzymatic activities of MMP-2 (lower band: 72 kDa) and MMP-9 (upper band: 95 kDa). (B) Densitometric analysis of MMP-2 ( $*p < 0.05$ ,  $n = 3$ ) and (C) MMP-9 normalization to cellularity.

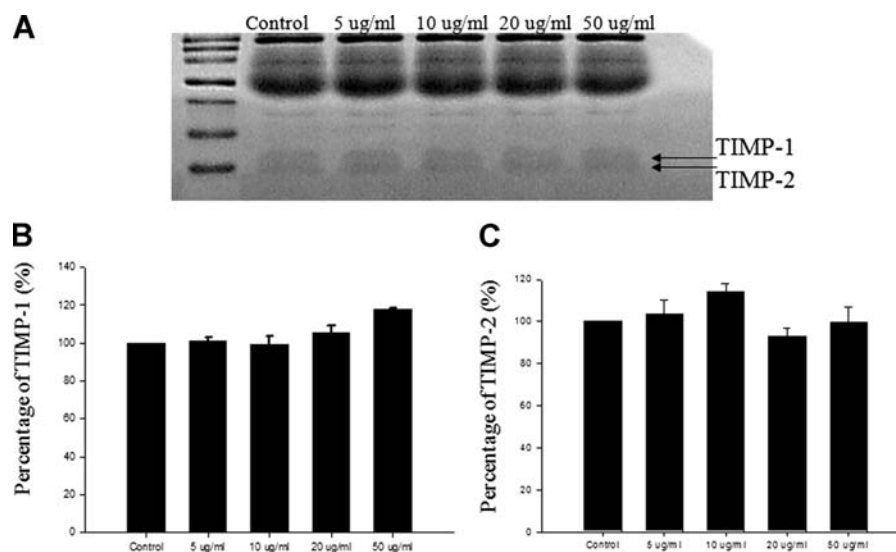
expression of MMP-2 at the edges of an acute tear in the supraspinatus tendon.<sup>26</sup> Besides, the expression of MMP-2 was up-regulated in Achilles tendinopathy.<sup>19,20</sup>

Previous study using canine tenocytes with treatment of ciprofloxacin has demonstrated an increase of matrix-degrading activity.<sup>5</sup> The present study further confirms that MMP-2 is up-regulated both in vitro and ex vivo after ciprofloxacin treatment. Collagenase which cleaves collagen at a single locus in the collagen triple helix, creating 3/4 and 1/4 fragments which can then be further degraded by a variety of proteinases including the gelatinases, such as MMP-2 and MMP-9.<sup>27</sup> A study of synovial fluids from the glenohumeral joint of patients with rotator cuff pathology showed no change in the levels of TIMP-1.<sup>28</sup> This study also revealed that

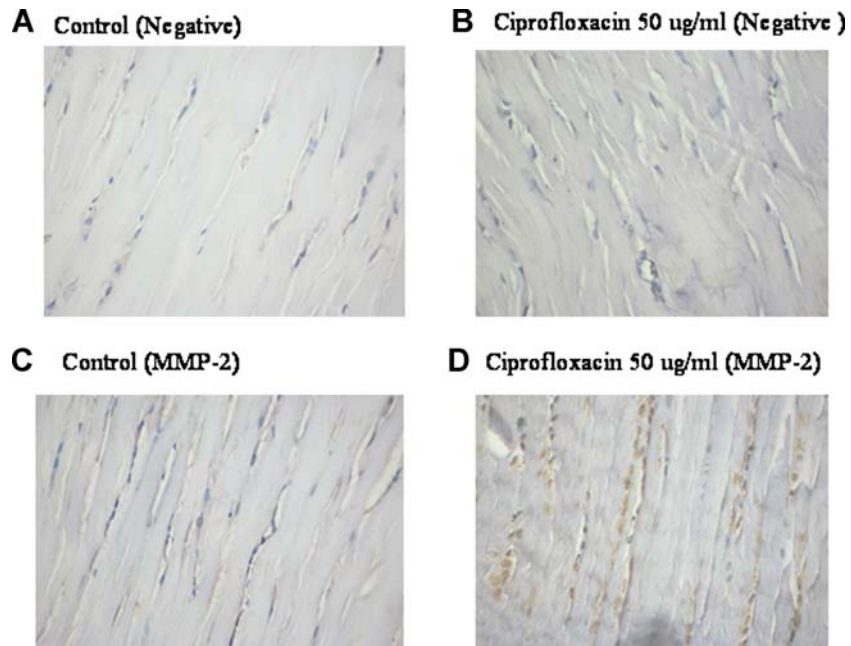
ciprofloxacin exerted no effect on TIMP-1 or TIMP-2 expression.

The results of this study implied that ciprofloxacin might exert a negative effect on tendon structure or its healing process through the mechanism by up-regulating gelatinase (MMP-2) expression. This finding is further supported by the result that decreased amount of type I collagen in the conditioned medium and increased degradation of type I collagen. Blockage of MMPs has been demonstrated to enhance tendon-bone healing of anterior cruciate ligament grafts that further supports the hypothesis of this study.<sup>29</sup>

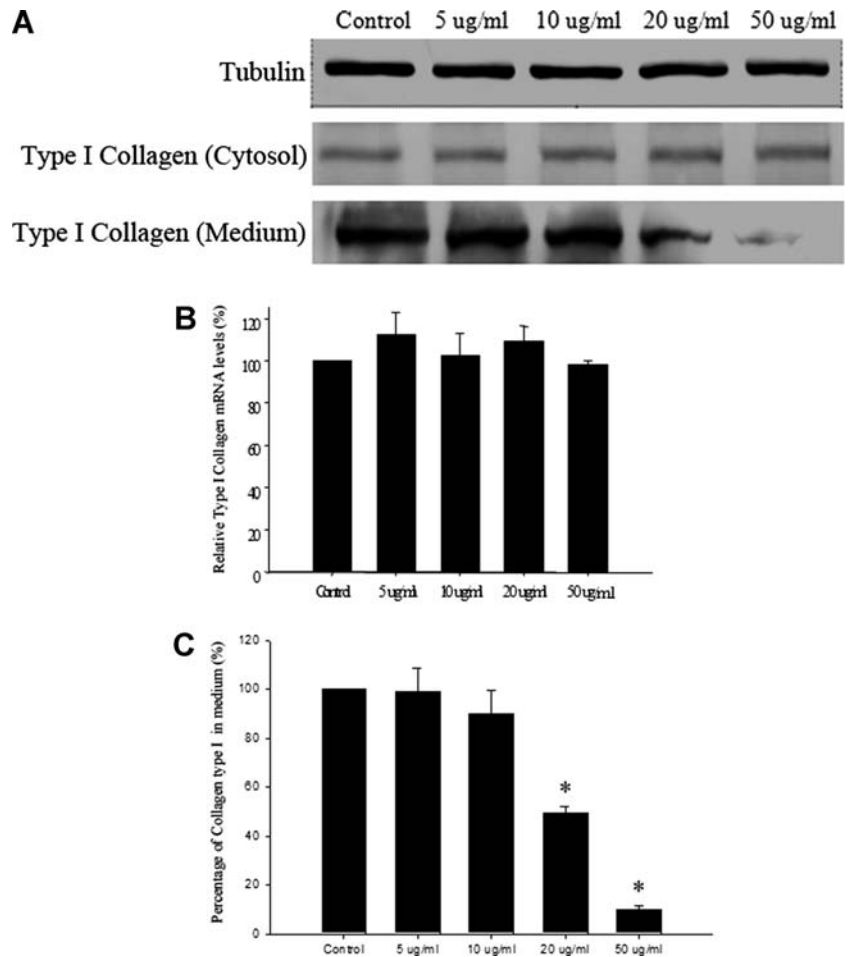
In studies of human tenocytes pretreated with interleukin-1 (IL-1), it revealed an up-regulation of MMP-1 and MMP-3 expression by ciprofloxacin.<sup>7</sup>



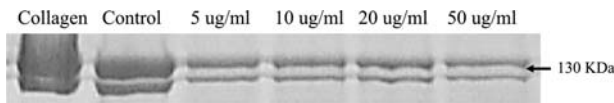
**Figure 3.** (A) Reverse zymography of the conditioned medium (CM) revealed that the enzymatic activities of TIMP-1 (28.5 kDa) and TIMP-2 (21 kDa). Densitometric analysis of TIMP-1 (B) and TIMP-2 (C) normalization to cell number (MTT results) revealed that the enzymatic activity was unchanged by ciprofloxacin ( $n = 3$ ).



**Figure 4.** Immunohistochemical staining for MMP-2. Tendon cells in tendon explants treated with 50 g/mL ciprofloxacin (D) revealing more brown staining within the cytoplasm as compared to tendon cells in control explants (C) (A and B, negative: negative control).



**Figure 5.** (A) Western blot analysis of tubulin (as an internal control) and type I collagen in cytosol and conditioned medium which were identified at 57 and 95 kDa, respectively. (B) Real-time RT-PCR revealed the mRNA expression of type I collagen. (C) Densitometric analysis of secreted type I collagen in conditioned medium normalization to cell number was decreased by ciprofloxacin (\* $p < 0.05$ ,  $n = 3$ ).



**Figure 6.** Type I collagen was degraded by conditioned medium from cells treated with ciprofloxacin. Two major bands revealed by Coomassie blue staining after nondenaturing gel electrophoresis were indicated by arrow.

As compared with this previous study, the present study documented that ciprofloxacin could directly up-regulate expressions of MMP-2 without the presence of IL-1. Furthermore, to our knowledge, the result of immunohistochemical staining for MMP-2 in this study is the first one to document the *ex vivo* effect of ciprofloxacin on up-regulating MMP-2. Besides, this study specifically documents that type I collagen might be degraded by MMPs which are up-regulated by ciprofloxacin. Because fluoroquinolones may also stimulate inflammatory pathways in or around the tendon,<sup>30</sup> the combined effect on tendon matrix turnover may account for the mechanisms of tendinopathy in some patients treated with ciprofloxacin.

An analysis of the results obtained from this study suggests that enhanced MMP expression might significantly compromise the integrity of the tendon ECM and thus induce the occurrence of tendinopathy or tendon rupture. The peak serum concentrations of ciprofloxacin given orally or intravenously were reported to range from 0.5 to 10  $\mu\text{g}/\text{mL}$ <sup>31–34</sup> and 5  $\mu\text{g}/\text{mL}$  ciprofloxacin was the initial concentration to induce the expressions of MMP-2. Although, the concentration of ciprofloxacin in tendon tissue after standard dosing regimens remains unknown, the result of this study suggest a potential link between ciprofloxacin-associated tendinopathy and increased dosage of ciprofloxacin.

Because of the small size of the explants (about 0.5 cm in length, 0.2 cm in diameter), it is difficult to perform a biomechanical test to evaluate the changes in mechanical properties of a tendon after ciprofloxacin treatment. Further animal studies to investigate if there is a deterioration of the mechanical properties of a tendon after ciprofloxacin treatment are needed to validate the findings of this study.

In conclusion, ciprofloxacin up-regulates the expression of MMP-2 in tendon cells with concomitant degradation of type I collagen. These findings provide novel molecular mechanisms of ciprofloxacin-induced tendinopathy or tendon rupture.

## ACKNOWLEDGMENTS

The author thank the National Science Council, Taiwan for financially support this research.

## REFERENCES

- Pierfitte C, Royer RJ. 1996. Tendon disorders with fluoroquinolones. *Therapie* 51:419–420.
- Zabraniecki L, Negrier I, Vergne P, et al. 1996. Fluoroquinolone induced tendinopathy: Reports of 6 cases. *J Rheumatol* 23:516–520.

- Kato M, Takada S, Kashida Y, et al. 1995. Histological examination on Achilles tendon lesions induced by quinolone antibacterial agents in juvenile rats. *Toxicol Pathol* 23:385–392.
- Shakibaei M, Stahlmann R. 2001. Ultrastructure of Achilles tendon from rats after treatment with fleroxacin. *Arch Toxicol* 75:97–102.
- Williams RJ, Attia E, Wickiewicz TL, et al. 2000. The effect of ciprofloxacin on tendon, paratenon, and capsular fibroblast metabolism. *Am J Sports Med* 28:364–369.
- Bernard-Beaubois K, Hecquet C, Hayem G, et al. 1998. In vitro study of cytotoxicity of quinolones on rabbit tenocytes. *Cell Biol Toxicol* 14:283–292.
- Corps AN, Harrall RL, Curry VA, et al. 2002. Ciprofloxacin enhances the stimulation of matrix metalloproteinase 3 expression by interleukin-1 $\beta$  in human tendon-derived cells: A potential mechanism of fluoroquinolone-induced tendinopathy. *Arthritis Rheum* 46:3034–3040.
- Tsai WC, Hsu CC, Tang FT, et al. 2008. Ciprofloxacin mediated cell proliferation inhibition and G2/M cell cycle arrest in tenocytes. *Arthritis Rheum* 58:1657–1663.
- Tsai WC, Hsu CC, Chen HC, et al. 2009. Ciprofloxacin-mediated inhibition of tenocyte migration and down-regulation of focal adhesion kinase phosphorylation. *Eur J Pharmacol* 607:23–26.
- O'Brien M. 1992. Functional anatomy and physiology of tendons. *Clin Sports Med* 11:505–520.
- Amiel D, Frank C, Harwood F, et al. 1984. Tendons and ligaments: A morphological and biochemical comparison. *J Orthop Res* 1:257–265.
- Duance VC, Restall DJ, Beard H, et al. 1977. The location of three collagen types in skeletal muscle. *FEBS Lett* 79:248–252.
- Maffulli N, Ewen SW, Waterston SW, et al. 2000. 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* 28:499–505.
- Jones GC, Corps AN, Pennington CJ, et al. 2006. Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human Achilles tendon. *Arthritis Rheum* 54:832–842.
- Nagase H, Woessner JF, Jr. 1999. Matrix metalloproteinases. *J Biol Chem* 274:21491–21494.
- Aimes RT, Quigley JP. 1995. Matrix metalloproteinase-2 is an interstitial collagenase. Inhibitor-free enzyme catalyzes the cleavage of collagen fibrils and soluble native type I collagen generating the specific 3/4- and 1/4-length fragments. *J Biol Chem* 270:5872–5876.
- Gomez DE, Alonso DF, Yoshiji H, et al. 1997. Tissue inhibitors of metalloproteinases: Structure, regulation and biological functions. *Eur J Cell Biol* 74:111–122.
- Goupille P, Jayson MIV, Valat J, et al. 1998. Matrix metalloproteinases: The clue to intervertebral disc degeneration? *Spine* 23:1612–1626.
- Alfredson H, Lorentzon M, Backman S, et al. 2003. cDNA-arrays and real-time quantitative PCR techniques in the investigation of chronic Achilles tendinosis. *J Orthop Res* 21:970–975.
- Karousou E, Ronga M, Vigetti D, et al. 2008. Collagens, proteoglycans, MMP-2, MMP-9 and TIMPs in human Achilles tendon rupture. *Clin Orthop Relat Res* 466:1577–1582.
- Tsai WC, Pang JH, Hsu CC, et al. 2006. Ultrasound stimulation of types I and III collagen expression of tendon cell and upregulation of transforming growth factor beta. *J Orthop Res* 24:1310–1316.

22. Gotoh M, Hamada K, Yamakawa H, et al. 1997. Significance of granulation tissue in torn supraspinatus insertions: An immunohistochemical study with antibodies against interleukin-1 beta, cathepsin D, and matrix metalloproteinase-1. *J Orthop Res* 15:33–39.
23. Fu SC, Chan BP, Wang W, et al. 2002. Increased expression of matrix metalloproteinase 1 (MMP1) in 11 patients with patellar tendinosis. *Acta Orthop Scand* 73:658–662.
24. Riley GP, Curry V, DeGroot J, et al. 2002. Matrix metalloproteinase activities and their relationship with collagen remodeling in tendon pathology. *Matrix Biol* 21:185–195.
25. Lo IK, Marchuk LL, Hollinshead R, et al. 2004. Matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase mRNA levels are specifically altered in torn rotator cuff tendons. *Am J Sports Med* 32:1223–1229.
26. Choi HR, Seiji K, Kazuyoshi H, et al. 2002. Expression and enzymatic activity of MMP-2 during healing process of acute supraspinatus tendon tear in rabbits. *J Orthop Res* 20:927–933.
27. Bigg HF, Rowan AD, Barker MD, et al. 2007. Activity of matrix metalloproteinase-9 against native collagen types I and III. *FEBS J* 274:1246–1255.
28. Yoshihara Y, Hamada K, Nakajima T, et al. 2001. Biochemical markers in the synovial fluid of glenohumeral joints from patients with rotator cuff tear. *J Orthop Res* 19:573–579.
29. Demirag B, Sarisozen B, Ozer O, et al. 2005. Enhancement of tendon-bone healing of anterior cruciate ligament grafts by blockage of matrix metalloproteinases. *J Bone Joint Surg Am* 87:2401–2410.
30. Kashida Y, Kato M. 1997. Characterization of fluoroquinolone-induced Achilles tendon toxicity in rats: Comparison of toxicities of 10 fluoroquinolones and effects of anti-inflammatory compounds. *Antimicrob Agents Chemother* 41:2389–2393.
31. Bergeron MG. 1989. The Pharmacokinetics and tissue penetration of the fluoroquinolones. *Clin Invest Med* 12:20–22.
32. Dan M, Golomb J, Gorea A, et al. 1986. Concentration of ciprofloxacin in human prostatic tissue after oral administration. *Antimicrob Agents Chemother* 30:88–89.
33. MacGown AP, White LO, Brown NM, et al. 1994. Serum ciprofloxacin concentrations in patients with severe sepsis being treated with ciprofloxacin 200 mg i.v. bd irrespective of renal function. *J Antimicrob Chemother* 33:1051–1054.
34. Shah A, Lettieri J, Kaiser L, et al. 1994. Comparative pharmacokinetics and safety of ciprofloxacin 400 mg i.v. thrice daily versus 750 mg po twice daily. *J Antimicrob Chemother* 33:795–801.