Free Radical Research, April 2009; 43(4): 323–328

informa
healthcare

The mitochondria targeted antioxidant MitoQ protects against fluoroquinolone-induced oxidative stress and mitochondrial membrane damage in human Achilles tendon cells

DAMON A. LOWES[1], CAROL WALLACE[1], MICHAEL P. MURPHY[2], NIGEL R. WEBSTER[1], & HELEN F. GALLEY[1]

[1]*Division of Applied Medicine, School of Medicine & Dentistry, University of Aberdeen, UK, and* [2]*MRC Dunn Human Nutrition Unit, Cambridge, UK*

(Received 30 September 2008; revised 6 January 2009)

Abstract
Tendinitis and tendon rupture during treatment with fluoroquinolone antibiotics is thought to be mediated via oxidative stress. This study investigated whether ciprofloxacin and moxifloxacin cause oxidative stress and mitochondrial damage in cultured normal human Achilles' tendon cells and whether an antioxidant targeted to mitochondria (MitoQ) would protect against such damage better than a non-mitochondria targeted antioxidant. Human tendon cells from normal Achilles' tendons were exposed to 0–0.3 mM antibiotic for 24 h and 7 days in the presence of 1 μM MitoQ or an untargeted form, idebenone. Both moxifloxacin and ciprofloxacin resulted in up to a 3-fold increase in the rate of oxidation of dichlorodihydrofluorescein, a marker of general oxidative stress in tenocytes ($p < 0.0001$) and loss of mitochondrial membrane permeability ($p < 0.001$). In cells treated with MitoQ the oxidative stress was less and mitochondrial membrane potential was maintained. Mitochondrial damage to tenocytes during fluoroquinolone treatment may be involved in tendinitis and tendon rupture.

Keywords: *Mitochondria, coenzyme Q10, MitoQ, idebenone, antibiotic, tendon*

Introduction

Tendinopathy is a degenerative process thought to be a preliminary phase to tendon rupture. There are several predisposing factors to tendinopathy [1] including excessive exercise, patient age/sex and endocrine factors. However, it has been proposed that the defective repair response predisposing to ineffective tendon healing in over-use tendinopathy is due to dysregulated reactive oxygen species production within the extracellular environment of the tendon [2].

Fluoroquinolone antibiotics are broad spectrum bactericidal agents which inhibit bacterial DNA gyrase and topisomerases and include ciprofloxacin, the first oral fluoroquinolone, introduced in 1987, and moxifloxacin, introduced in 2000. Fluoroquinolone antibiotics are prescribed for a wide range of infections, including respiratory, gastrointestinal and genito-urinary tract infections and account for $\sim$40% of the antibiotic market in the USA alone.

Many of the older fluoroquinolones have been found to induce oxidative stress and antioxidant depletion in cultured rabbit tendon cells [3,4] and this is thought to contribute to the high incidence of tendon rupture in patients taking fluoroquinolone antibiotics [2,5–8]. Warnings about the risk of tendon rupture and tendonitis have appeared in the package inserts for these drugs for the past 10 years and a call has been made for this to be upgraded to a so-called 'black box' warning [9]. There are over 250 reports of tendon ruptures linked to fluoroquinolone use in the Food and Drug Administration (FDA) adverse event database between 1997–2006.

Correspondence: Dr Helen Galley, Division of Applied Medicine, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB41 8TJ, UK. Tel: +44 (0) 1224 552775. Fax: +44 (0) 1224 555766. Email: h.f.galley@abdn.ac.uk

ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760902736275

Mitochondria are a major source and target of oxidative stress and, in the face of inadequate antioxidant protection, this results in mitochondrial dysfunction, which may contribute to tendinopathy. Oxidative damage to mitochondria in chondrocytes and tenocytes induced by fluoroquinolones has been reported [10], which may have consequences for cell function and integrity. Ciprofloxacin and moxifloxacin have differential modulatory effects on inflammatory responses [11,12]. However, the effect of moxifloxacin in terms of oxidative stress in human tendon cells has not been reported, although there is evidence of moxifloxacin causing tendinopathy [13].

Coenzyme Q10 (CoQ10) is the predominant form of the electron carrier and antioxidant ubiquinone in man and has an important role in protecting mitochondria against oxidative stress. Lipophilic cations, such as triphenyl-phosphonium (TPP), accumulate in mitochondria several hundred-fold via the large membrane potential (negative inside) generated by mitochondria during oxidative phosphorylation [14]. This property has been exploited by covalently attaching decyl ubiquinone to the TPP cation, generating mitochondria-targeted ubiquinone (MitoQ) which is selectively accumulated within mitochondria [15–18].

The majority of the tendon ruptures reported in patients on fluoroquinolone antibiotics involve the Achilles' tendon [1,8,13]. We determined whether moxifloxacin and ciprofloxacin treatment induced oxidative stress and mitochondrial dysfunction in cultured human Achilles' tendon cells *in vitro* and whether MitoQ and an untargeted form of ubiquinone, idebenone, were protective.

Materials and methods

Cell culture

Tendon cells were a generous gift from Professor Richard Aspden of the Bone and Musculoskeletal Research Group, University of Aberdeen. Following Ethical Committee approval and written informed consent, tendons were harvested by collagenase digestion from normal human Achilles' tendons obtained from amputated legs, as previously described in detail [19]. Following resuscitation from frozen storage in liquid nitrogen, cells were cultured in 75 cm^2 flasks containing Ham's F-12 medium supplemented with 20% foetal calf serum, 2 mM glutamine and 0.2 μg/ml insulin, at 37 °C in a moist atmosphere with 5% CO_2 and were used for experiments at passage 2, to avoid any phenotypic drift [20]. Phenotype was established by analysing type I collagen, decorin and integrin-1β expression [20].

Ciprofloxacin and moxifloxacin were a kind gift from Bayer Plc (Newbury, UK). Confluent cell monolayers were exposed to 0–0.3 mM concentrations of ciprofloxacin or moxifloxacin with either 1 μM MitoQ mesylate, 1 μM idebenone or 1 μM decylTPP bromide salt (dTPP) as control (Antipodean Pharmaceuticals, San Francisco, CA). The concentrations of antibiotics were chosen to mimic the range of intracellular therapeutic levels achieved *in vivo*. Ciprofloxacin and moxifloxacin were prepared in sterile water and MitoQ, dTPP and idebenone were prepared in ethanol. Appropriate solvent controls were used. A sub-lethal concentration of hydrogen peroxide (200 μM) was used as a positive control.

Oxidative stress

For measurement of general oxidative stress, resuscitated cells were grown in 96 well plates at a density of 5000 cells/well for 24 h before experimentation. The cells were loaded with the probe 5-(6)-carboxy- 2,7'-dichlorodihydrofluorescein-diacetate (DCF, 50 μM) for 60 min then washed twice in phosphate buffered saline (PBS). Antibiotics plus dTPP, MitoQ or idebenone were then added as described above. The rate of formation of the fluorescent derivative 5-(6)-carboxy-2,7'-dichlorofluorescein, as a result of oxidation by intracellular radicals, was measured with a FLUOstar Optima spectrofluorimeter (BMG Lab Technologies, Germany) at 37°C over 24 h at an excitation wavelength of 485 nm and emission wavelength of 530 nm [21].

Mitochondrial membrane potential

For measurement of mitochondrial membrane potential, cells were again grown in 96 well plates and cultured with antibiotics plus dTPP, MitoQ or idebenone. JC-1 dye (5,5', 6,6'tetrachloro-1,1',3,3'-tetraethyl-benzimidazolcarbocyanine iodide) is a lipophilic cation which accumulates in intact mitochondria and fluoresces when excited at 490 nm. After culture periods of up to 7 days, JC-1 (10 μg/ml) was added to each well and cells were incubated in the dark at 37°C for 20 min. The cells were then washed twice in PBS and fluorescence was measured at 590 nm. Decreased fluorescence is indicative of loss of mitochondrial membrane potential [22,23]. As a positive control, cells were exposed to 5 μM rotenone for 5 h prior to addition of JC-1.

Viable cell number was measured using acid phosphatase activity [24]. Briefly, conditioned media was removed and cells were washed with PBS. Acid phosphatase buffer, containing 0.1% triton X-100, 0.1 M sodium acetate pH 5.0 and 5 mM p-nitrophenyl phosphate (100 μl) was added to each well and cells were incubated at 37°C for 1 h. The reaction was stopped by the addition of 20 μl 1 M NaOH and absorbance was measured at 405 nm. DCF oxidation rate and JC-1 fluorescence were normalized to viable cell number.

Statistical analysis

Data are presented as median and interquartile range from seven treatment replicates from three separate experiments using cells from three different tendon donors for each experiment. No assumptions have been made about the distribution of the data, which were compared using Kruskal Wallis with *post hoc* testing using the Mann Whitney U-test. A p-value of < 0.05 was taken to be significant.

Results

Cell viability

There was a dose-dependent loss in viable cell number in cells exposed to antibiotics ($p < 0.0001$), whereas viable cell count was maintained when cells were also exposed to MitoQ and to a lesser extent by idebenone (Figure 1).

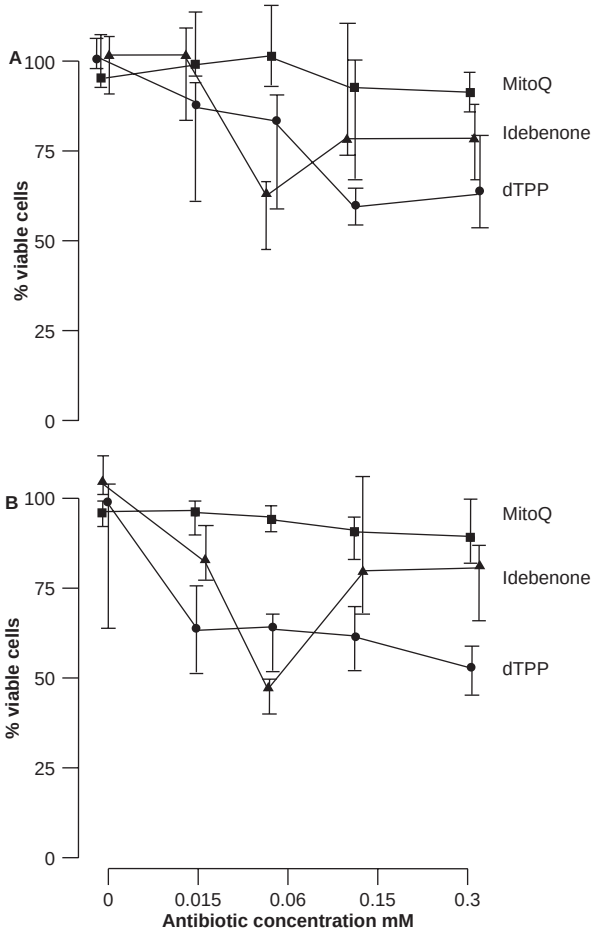

Figure 1. Percentage viable cells as determined by acid phosphatase activity in (A) ciprofloxacin-treated human Achilles' tendon cells and (B) moxifloxacin-treated cells. Cells were incubated at 37 °C for 1 h in acid phosphatase buffer following incubation for 24 h with either 0–0.3 mM ciprofloxacin or moxifloxain plus 1 μM MitoQ, dTPP or idebenone. The reaction was stopped with NaOH and absorbance was measured at 405 nm. Values are median and interquartile range of seven replicate measurements from each of three different tendon donors. See text for p-values. Circles are dTPP, squares are MitoQ and triangles are idebenone- treated cells.

Oxidative stress

To assess whether oxidative stress occurred upon antibiotic treatment, we treated tendon cells with a range of concentrations of ciprofloxacin or moxifloxacin. The actual rate of DCF oxidation varied between tendon donor and so results are shown as percentage change in DCF oxidation compared to solvent control cells without antibiotic (Figures 1 and 2).

The DCF oxidation rate increased with increasing ciprofloxacin or moxifloxacin dose (both $p < 0.0001$, Figures 2 and 3) in solvent control cells such that at the highest antibiotic concentration the rate was 1–3-fold greater than in cells without antibiotic (median 164%; IQ range 128–200% of control at 0.3 mM ciprofloxacin and 208%, 185–308% at 0.3 mM moxifloxacin, $p = 0.05$ and $p = 0.007$, respectively, Figures 2 and 3).

When cells were also treated with 1 μM MitoQ, the increase in DCF oxidation rate was prevented in both ciprofloxacin and moxifloxacin-treated cells. The median fluoresence change with MitoQ plus 0.3 mM ciprofloxacin was 97% (IQ range 88–98%) of control and 95% (IQ range 86–105%) of control with MitoQ plus 0.3 mM moxifloxacin (both $p > 0.05$). The untargeted form of ubiquinone, idebenone, reduced

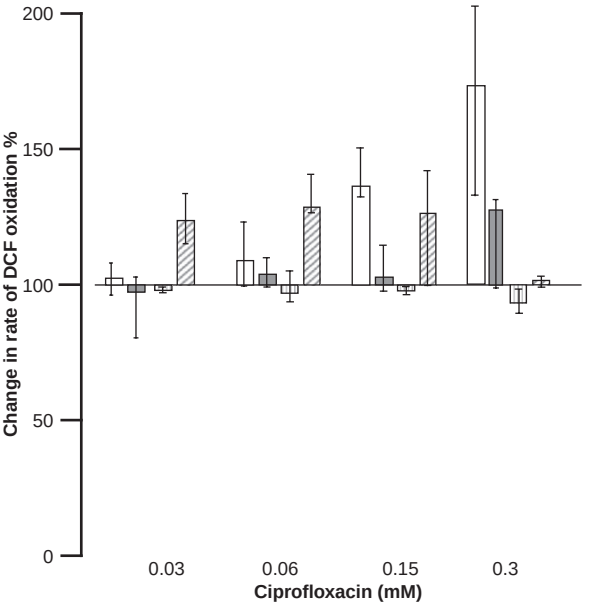

Figure 2. Change in the rate of oxidation of dichlorodihydrofluorescein (DCF) in human Achilles' tendon cells treated with ciprofloxacin compared to cells without antibiotic and in the presence of MitoQ, dTPP or idebenone. Cells were loaded with the DCF probe for 60 min, washed and 0–0.3 mM ciprofloxacin plus 1 μM dTPP, MitoQ or idebenone was added. The rate of formation of the fluorescent derivative of DCF as a result of oxidation by intracellular radicals was measured at 37 °C over 24 h and expressed as the percentage of the rate in solvent control cells. Values are median and interquartile range of seven replicate measurements from each of three different tendon donors. See text for p-values. □ Control; ■ dTPP; ▥ MitoQ; ▨ Idebenone.

DCF oxidation rate to some extent but was less effective than MitoQ (Figures 2 and 3). DCF oxidation rate remained high in cation carrier control-treated (dTPP) cells ($p < 0.05$, Figures 2 and 3). Hydrogen peroxide caused an ~3-fold increase in DCF oxidation (data not shown).

Mitochondrial membrane potential

As a measure of the effect of ciprofloxacin and moxifloxacin on mitochondrial function, we assessed the effects on mitochondrial membrane potential as determined by JC-1 fluorescence. The positive control rotenone caused an almost complete loss of JC-1 fluorescence (data not shown).

Increasing concentrations of ciprofloxacin or moxifloxacin caused decreased JC-1 fluorescence, i.e. decreased mitochondrial membrane potential after 7 days, compared to cells without antibiotics (both $p < 0.001$, Figures 4 and 5). At the highest concentration of ciprofloxacin (0.3 mM) median [IQ range] JC-1 fluorescence was 21 [19–26] $\times 10^3$ units compared to 39 [37–42] $\times 10^3$ units without antibiotics ($p = 0.04$, Figure 4). In cells treated with 0.3 mM moxifloxacin JC-1 fluorescence was 13 [10–20] $\times 10^3$ units compared to 31 [29–34] $\times 10^3$ units without antibiotics ($p = 0.02$, Figure 5).

In cells treated with MitoQ, mitochondrial membrane potential was maintained or even augmented. In cells treated with MitoQ and 0.3 mM ciprofloxacin, JC-1 fluorescence was 68 [63–80] $\times 10^3$ units ($p < 0.0001$ compared to without MitoQ) and in cells treated with MitoQ and 0.3 mM moxifloxacin, JC-1 fluorescence was 61 [58–70] $\times 10^3$ units ($p < 0.0001$ compared to without MitoQ, Figures 4 and 5). This large increase in JC-1 fluorescence was not seen when cells were treated with either dTPP or idebenone plus antibiotic. MitoQ alone, without antibiotic, caused an increase in JC-1 fluoresence in some experiments but not others. There was no change in membrane potential at 24 h (data not shown).

Discussion

We have shown that both moxifloxacin and ciprofloxacin caused a decrease in cell viability coupled with an increase in DCF oxidation rate, resulting in altered JC-1 fluoresence indicative of mitochondrial membrane damage, in tendon cells from normal human Achilles' tendons *in vitro*. We have also shown that the mitochondria targeted antioxidant MitoQ prevents ciprofloxacin- and moxifloxacin-induced oxidative stress and loss of mitochondrial membrane

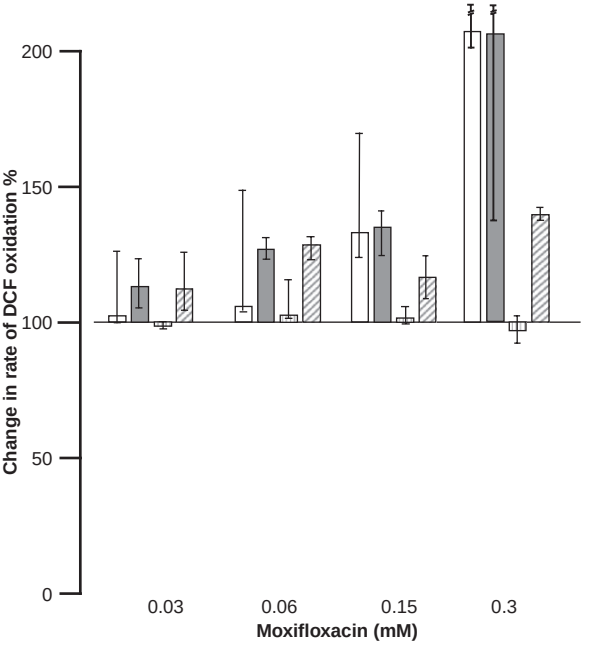

Figure 3. Change in the rate of oxidation of dichlorodihydrofluorescein (DCF) in human Achilles' tendon cells treated with moxifloxacin compared to cells without antibiotic and in the presence of MitoQ, dTPP or idebenone. Cells were loaded with the DCF probe for 60 min, washed and 0–0.3 mM moxifloxacin plus 1 μM dTPP, MitoQ or idebenone was added. The rate of formation of the fluorescent derivative of DCF as a result of oxidation by intracellular radicals was measured at 37 °C over 24 h and expressed as the percentage of the rate in solvent control cells. Values are median and interquartile range of seven replicate measurements from each of three different tendon donors. See text for *p*-values. □ Control; ■ dTPP; ▥ MitoQ; ▨ Idebenone.

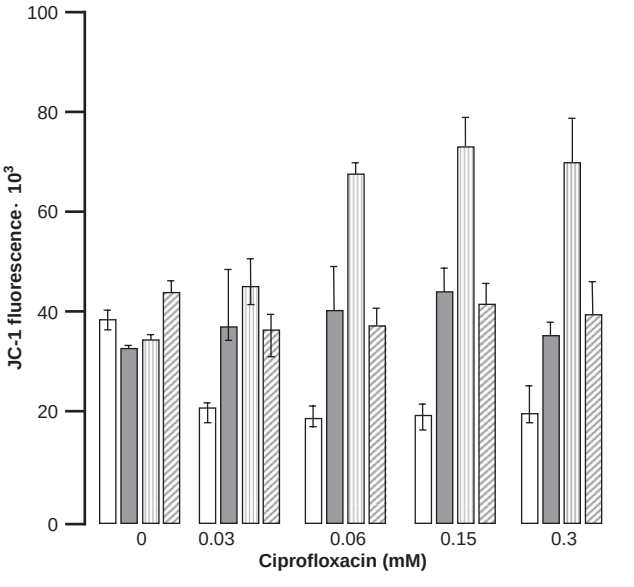

Figure 4. Mitochondrial membrane potential measured as JC-1 fluorescence in human Achilles' tendon cells treated with ciprofloxacin compared to cells without antibiotic and in the presence of either MitoQ, dTPP or idebenone. After 24 h culture with 0–0.3 mM ciprofloxacin plus 1 μM either MitoQ, dTPP or idebenone, JC-1 was added and cells were incubated at 37 °C for 20 min and fluorescence was measured at 590 nm. Values are median and interquartile range of seven replicate measurements from each of three different tendon donors. See text for *p*-values. □ Control; ■ dTPP; ▥ MitoQ; ▨ Idebenone.

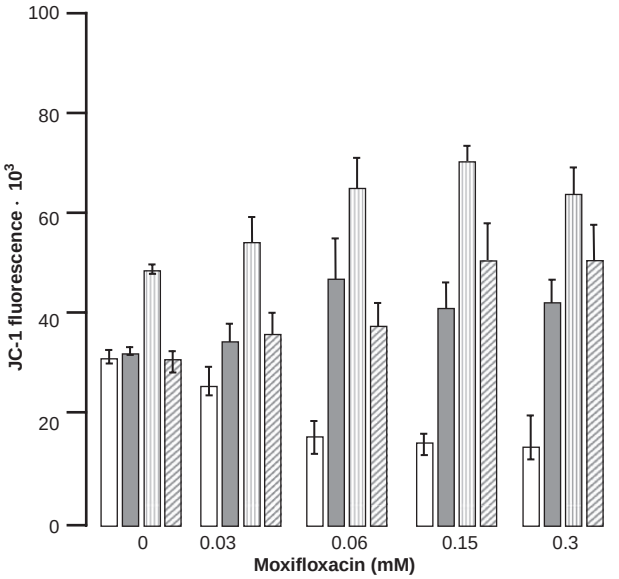

Figure 5. Mitochondrial membrane potential measured as JC-1 fluorescence in human Achilles' tendon cells treated with moxifloxacin compared to cells without antibiotic and in the presence of either MitoQ, dTPP or idebenone. After 24 h culture with 0–0.3 mM moxifloxacin plus 1 μM either MitoQ, dTPP or idebenone, JC-1 was added and cells were incubated at 37 °C for 20 min and fluorescence was measured at 590 nm. Values are median and interquartile range of seven replicate measurements from each of three different tendon donors. See text for *p*-values. □ Control; ■ dTPP; ▥ MitoQ; ▨ Idebenone.

potential and an untargeted form of ubiquinone is less effective.

Previous studies using other cells have also shown a protective effect of MitoQ against oxidative stress. Treatment of bovine aortic endothelial cells with MitoQ protected cells from peroxide-induced oxidative stress and maintained mitochondrial function [17]. In studies using fibroblasts from patients with Friedreich's ataxia, MitoQ prevented cell death after *in vitro* glutathione depletion and was several hundred-fold more effective than the untargeted form of ubiquinone, idebenone [18]. We have also very recently reported that MitoQ, but not untargeted forms of ubiquinone, protect human endothelial cells against oxidative stress and modulate cytokine release in a model of sepsis [25].

Fluorinated-4-quinolones, including ciprofloxacin and moxifloxacin, are nalidixic acid analogue antibiotics, which exert their bactericidal effect by inhibiting DNA gyrase activity. Tendinitis and tendon rupture have been reported to occur in ~1% of patients treated with fluoroquinolones. Experiments in animals showed that fluorinated quinolones can damage cartilage and tendons [10] and it was suggested on the basis of histological evidence that damage may occur as a result of mitochondrial injury [26]. The fluoroquinolones pefloxacin and norfloxacin both caused decreased mitochondrial enzyme activity in rabbit tendon cells [4]. Another study reported increased oxidative stress coupled with oxidation of important intracellular redox couples such as glutathione in a rabbit tendon cell line exposed to ciprofloxacin, pefloxacin, ofloxacin or levofloxacin [27]. This damage was less in cells treated with the antioxidant anethole dithiolethione [28]. Others reported that ciprofloxacin-induced oxidative damage was partially prevented by vitamin E in fibroblasts or astrocytes *in vitro* [29–31] and by vitamin E or allopurinol in liver and brain from rats [32] and in mice, pefloxacin-induced oxidative damage was prevented by administration of N-acetylcysteine [33]. Thus, ciprofloxacin induces oxidative stress in various cell types including animal tendon cells. However, although moxifloxacin has been implicated as a cause of tendinitis in a case report [13], there have been no reports of moxifloxacin-induced oxidative stress. We also found no reports of fluoroquinolone antibiotics mediating mitochondrial damage in human tendon cells and no information on whether antioxidant protection of mitochondria can prevent such damage. In this study we found that both ciprofloxacin and moxifloxacin caused concentration-dependent increases in radical production resulting in loss of mitochondrial membrane potential and that the mitochondria targeted antioxidant MitoQ was protective. Treatment of antibiotic exposed cells with non-mitochondria targeted idebenone did not protect the cells to the same extent.

These results show that both ciprofloxacin and moxifloxacin cause oxidative stress and mitochondrial membrane damage which may contribute to the development of tendinitis and tendon rupture in some patients. Our data also show that an antioxidant targeted to mitochondria can protect against this damage. Although theoretically MitoQ may be able to react with DCF, this probe is mainly in the cytosol as it cannot enter mitochondria after deacetylation. In contrast MitoQ remains mainly in the mitochondria [34] and therefore it is very unlikely that there is a direct interaction between MitoQ and DCF. Further experiments using additional measures of oxidative stress would be required to confirm this. However it is possible that MitoQ treatment may be of value in patients undergoing antibiotic treatment with fluoroquinolone antibiotics who are at increased risk of tendinitis. Oral MitoQ treatment for up to a year has been tested in two Phase II trials, in patients with hepatitis C and patients with Parkinson's disease, showing that it was well tolerated, with no serious adverse events.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Maffulli N, Kader D. Tendinopathy of tendon achillis. J Bone Joint Surg 2002;84B:1–8.

[2] Bestwick CS, Maffulli N. Reactive oxygen species and tendinopathy: do they matter? Br J Sports Med 2004;38: 672–674.

[3] Goswami M, Mangoli SH, Jawali N. Involvement of reactive oxygen species in the action of ciprofloxacin against Escherichia coli. Antimicrob Agents Chemother 2006;50:949–954.

[4] Pouzaud F, Bernard-Beaubois K, Thevenin M, Warnet J-M, Hayem G, Rat P. *In vitro* discrimination of fluoroquinolone toxicity on tendon cells: involvement of oxidative stress. J Pharmacol Exp Ther 2003;308:394–402.

[5] Shortt P, Wilson R, Erskine I. Tendinitis: the Achilles heel of quinolones! Emerg Med J 2006;23:e63.

[6] Corrao G, Zambon A, Bertu L, Mauri A, Paleari V, Rossi C, Venegoni M. Evidence of tendinitis provoked by fluoroquinolone treatment: a case-control study. Drug Saf 2006;29: 889–896.

[7] Burkhardt O, Kohnlein T, Pap T, Welte T. Recurrent tendinitis after treatment with two different fluoroquinolones. Scand J Infect Dis 2004;36:315–316.

[8] Ozaras R, Mert A, Tahan V, Uraz S, Ozaydin I, Yilmaz MH, Ozaras N. Ciprofloxacin and Achilles' tendon rupture: a causal relationship. Clin Rheumatol 2003;22:500–501.

[9] Reinberg S. FDA mandates Black Box Warning for some antibiotics. Available online at: http://www.medicinenet.com/script/main/art.asp?articlekey=90881, accessed 10 August 2008.

[10] Bernard-Beaubois K, Hecquet C, Hayem G, Rat P, Adolphe M. *In vitro* study of cytotoxicity of quinolones on rabbit tenocytes. Cell Biol Toxicol 1998;14:283–292.

[11] Williams AC, Galley HF, Webster NR. Differential effects of three antibiotics on T helper cell cytokine expression. J Antimicrob Chemother 2005;56:502–506.

[12] Werber S, Shalit I, Fabian I, Steuer G, Weiss T, Blau H. Moxifloxacin inhibits cytokine-induced MAP kinase and NF-kappaB activation as well as nitric oxide synthesis in a human respiratory epithelial cell line. J Antimicrob Chemother 2005; 55:293–300.

[13] Burkhardt O, Köhnlein T, Pap T, Welte T. Recurrent tendinitis after treatment with two different fluoroquinolones. Scand J Infect Dis 2004;36:315–316.

[14] Ross MF, Prime TA, Abakumova I, James AM, Porteous CM, Smith RA, Murphy MP. Rapid and extensive uptake and activation of hydrophobic triphenylphosphonium cations within cells. Biochem J 2008;411:633–645.

[15] James AM, Sharpley MS, Manas AR, Frerman FE, Hirst J, Smith RA, Murphy MP. Interaction of the mitochondria-targeted antioxidant MitoQ with phospholipid bilayers and ubiquinone oxidoreductases. J Biol Chem 2007;282: 14708–14718.

[16] James AM, Cocheme HM, Smith RAJ, Murphy MP. Interactions of mitochondria targeted and untargeted ubiquinones with the mitochondrial respiratory chain and reactive oxygen species: implications for the use of exogenous ubiquinones as therapies and experimental tools. J Biol Chem 2005;280: 21295–21312.

[17] Esplugues JV, Rocha M, Nunez C, Bosca I, Ibiza S, Herance JR, Ortega A, Serrador JM, D'Ocon P, Victor VM. Complex I dysfunction and tolerance to nitroglycerin: an approach based on mitochondrial targeted antioxidants. Circ Res 2006;99: 1067–1075.

[18] Jauslin ML, Meier T, Smith RAJ, Murphy MP. Mitochondria targeted antioxidants protect Friedreich ataxia fibroblasts from endogenous oxidative stress more effectively than untargeted antioxidants. FASEB J 2003;17:1972–1974.

[19] Maffulli N, Ewan SWB, Waterston SW, Reaper J, Barrass V. Tenocytes from ruptured and tendinopathic Achilles' tendon produced greater quantities of Type II collagen than tenocytes from normal Achilles' tendons. Am J Sports Med 2000;8: 499–505.

[20] Yao L, Bestwick CS, Bestwick LA, Maffulli N, Aspden RM. Phenotypic drift in human tenocyte culture. Tiss Eng 2006;12:1843–1849.

[21] Qin L, Block ML, Liu Y, Bienstock RJ, Pei Z, Zhang W, Wu X, Wilson B, Burka T, Hong JS. Microglial NADPH oxidase is a novel target for femtomolar neuroprotection against oxidative stress. FASEB J 2005;19:550–557.

[22] Reers M, Smith TW, Chen LB. J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. Biochemistry 1991;30:4480–4486.

[23] Salvioli S, Ardizzoni A, Franceschi C, Cossarizza A. JC-1, but not DiOC6(3) or rhodamine 123, is a reliable fluorescent probe to assess delta psi changes in intact cells: implications for studies on mitochondrial functionality during apoptosis. Febs Lett 1997;41:77–82.

[24] Yang TT, Sinai P, Kain SR. An acid phosphatase assay for quantifying the growth of adherent and nonadherent cells. Anal Biochem 1996;241:103–108.

[25] Lowes DA, Thottakam BMV, Murphy MP, Webster NR, Galley HF. The mitochondria-targeted antioxidant MitoQ protects against organ damage in a lipopolysaccharide-peptidoglycan model of sepsis. Free Radic Biol Med 2008;45: 1559–1565.

[26] Takayama S, Hirohashi M, Kato M, Shimada H. Toxicity of quinolone antibacterial agents. J Toxicol Environ Health 1995;45:1–45.

[27] Pouzaud F, Dutot M, Martin C, Debray M, Warnet JM, Rat P. Age-dependent effects on redox status, oxidative stress, mitochondrial activity and toxicity induced by fluoroquinolones on primary cultures of rabbit tendon cells. Comp Biochem Physiol C Toxicol Pharmacol 2006;143:232–241.

[28] Pouzaud F, Christen MO, Warnet JM, Rat P. Anethole dithiolethione: an antioxidant agent against tenotoxicity induced by fluoroquinolones. Pathol Biol 2004;52:308–313.

[29] Gürbay A, Garrel C, Osman M, Richard MJ, Favier A, Hincal F. Cytotoxicity in ciprofloxacin-treated human fibroblast cells and protection by vitamin E. Hum Exp Toxicol 2002;21: 635–641.

[30] Gürbay A, Gonthier B, Signorini-Allibe N, Barret L, Favier A, Hincal F. Ciprofloxacin-induced DNA damage in primary culture of rat astrocytes and protection by Vitamin E. Neurotoxicology 2006;27:6–10.

[31] Gürbay A, Gonthier B, Barret L, Favier A, Hincal F. Cytotoxic effect of ciprofloxacin in primary culture of rat astrocytes and protection by Vitamin E. Toxicology 2007;229: 54–61.

[32] Gurbay A, Hincal F. Ciprofloxacin-induced glutathione redox status alterations in rat tissues. Drug Chem Toxicol 2004;27: 233–242.

[33] Simonin MA, Gegout-Pottie P, Minn A, Gillet P, Netter P, Terlain B. Pefloxacin-induced achilles tendon toxicity in rodents: biochemical changes in proteoglycan synthesis and oxidative damage to collagen. Antimicrob Agents Chemother 2000;44:867–872.

[34] Ross MF, Prime TA, Abakumova I, James AM, Porteous CM, Smith RA, Murphy MP. Rapid and extensive uptake and activation of hydrophobic triphenylphosphonium cations within cells. Biochem J 2008;411:633–645.

This paper was first published online on iFirst on 23 February 2009.