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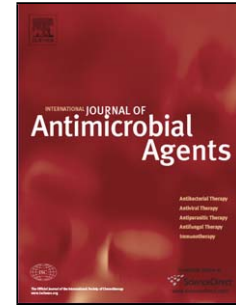
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Synergistic effects of dexamethasone and quinolones on human-derived tendon cells

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ABSTRACT

Quinolones and glucocorticoids are frequently used drugs that may cause tendinopathy as a rare adverse effect. We exposed human tenocyte cultures to the steroid dexamethasone alone or in combination with either ciprofloxacin or levofloxacin at concentrations of 3 mg/L and 10 mg/L. At concentrations corresponding to peak levels in plasma and tissues during therapy (ca. 3–10 mg/L), ciprofloxacin caused a significant decrease in collagen type I and the β_1 -integrin receptor. In contrast, no corresponding effect was induced by 3 mg/L levofloxacin. With both quinolones at 3 mg/L and 10 mg/L, the amount of matrix metalloproteinase-1 (MMP-1) and MMP-13 was increased. In addition, 3 mg/L ciprofloxacin and 10 mg/L levofloxacin activated caspase-3. Apoptotic changes were confirmed by electron microscopy. Incubation of human tenocytes with dexamethasone decreased the main matrix protein collagen type I, the transmembrane β_1 -integrin receptor and the cytoskeleton protein vinculin, but only at the high concentrations tested (0.1 μ M or 10 μ M). Concentrations of 0.1 μ M and 10 μ M dexamethasone increased the amount of MMPs and activated caspase-3 as an indicator of apoptosis. Combined exposure to quinolones and dexamethasone led to more pronounced effects in tenocyte cultures at most of the analysed endpoints. The clinical observations of an increased risk of quinolone-induced tendinopathy by glucocorticoids are supported by these in vitro data.

1. Introduction

Glucocorticoids and quinolones are commonly used agents for ambulant or inpatient therapy. The steroids are used systemically for several acute and chronic diseases such as chronic bronchopulmonary diseases as well as for immunosuppression following organ transplantation. The use of quinolones has increased during recent years because of the increasing resistance of pneumococci and other bacteria to β -lactams and macrolides [1]. Tendinopathies are rare but severe side effects that have been observed for both classes of drugs [2–10]. The term ‘tendinopathy’ comprises several forms of tendon damage from a reversible inflammation (tendinitis) up to tendon rupture requiring surgical intervention. Several case reports and retrospective studies indicate that the risk for tendinopathy is increased by concomitant use of glucocorticoids and quinolones [11–14]. Little is known about cellular alterations as an underlying cause of this adverse effect.

Tendons possess high tensile strength, mechanic stability and elastic properties. These characteristics are mediated by their extracellular matrix predominantly consisting of collagen type I fibrils and additional elastic fibres [15]. Embedded in this network of fibrils are tenocytes. These cells produce all components of the extracellular matrix and consequently play an important role in the structure and function of the tendon [16,17]. Cell–cell and cell–matrix interactions are mediated via transmembrane signal transduction receptors such as β_1 -integrins. They activate different signal transduction pathways, such as the mitogen-activated protein (MAP) kinase pathway, and thus control proliferation, differentiation and survival of tenocytes [18,19]. Undisturbed cell–matrix interactions are essential for cell viability, and inhibition of the MAP kinase pathway can induce programmed cell death [20,21].

We have previously described the effects of quinolones on tenocytes in vivo and in vitro. In vivo data have shown that tendons of quinolone-treated rats and dogs exhibited morphological and biochemical changes in the extracellular matrix and β_1 -integrins [22–24]. In vitro experiments with quinolone-incubated human-derived tenocytes revealed possible mechanisms in more detail. We found changes in the extracellular matrix, β_1 -integrin receptors as well as in signalling proteins of the MAP kinase pathway. In addition, probably as a consequence of the cell–cell and cell–matrix disturbance, apoptosis was induced in human tenocytes at concentrations of quinolones that are achieved in human plasma and tissues during therapy with these drugs [25].

Experimental studies performed to elucidate glucocorticoid-induced tendinopathies are scarce and somewhat controversial. In vitro studies revealed degenerative changes, reduced synthesis of collagen type I and proteoglycans, as well as decreased cell proliferation, viability and metabolism following glucocorticoid exposure [26–30]. Experimental evidence on the effects of steroids in combination with quinolones is even more limited. The in vitro experiments described here were conducted to study in more detail the effects of a glucocorticoid on human-derived tenocytes alone and in combination with a quinolone. Concentrations tested were orientated at maximum concentrations achievable in plasma during therapy.

2. Materials and methods

2.1. Cell culture

Finger tendon explants obtained during surgery were used to isolate primary human tenocytes [25]. Briefly, following non-enzymatic isolation, cells were expanded in monolayers and multiplied. Tenocytes were then pre-cultured on Petri dishes for 24 h at 37 °C in a humidified atmosphere with 5% CO₂ before incubation for 1–4 days with dexamethasone and/or a quinolone under the same conditions. The growth medium was composed of growth medium Ham's F-12 and Dulbecco's MEM (50:50) supplemented with 5% fetal calf serum (FCS). For incubation, the growth medium was supplemented with: 0.1 nM (ca. 0.039 µg/L), 1 nM (ca. 0.39 µg/L), 0.1 µM (ca. 39 µg/L) or 10 µM (ca. 3925 µg/L) dexamethasone; or 3 mg/L or 10 mg/L of either ciprofloxacin or levofloxacin; or 3 mg/L quinolone plus 0.1 nM dexamethasone; or 10 mg/L quinolone plus 1 nM dexamethasone. Controls were cultured in growth medium without addition of a quinolone or dexamethasone for 1–4 days. Samples for Western blotting were collected after 1, 2, 3 and 4 days of drug exposure, and samples for electron microscopy were collected after a 4-day drug exposure only.

2.2. Antibodies, drugs and culture medium

Polyclonal (rabbit anti-bovine) anti-collagen type I (AB749) antibody and alkaline phosphatase-conjugated sheep anti-mouse as well as sheep anti-rabbit secondary antibodies (AP303A, AP304A) were purchased from Chemicon International, Inc. (Temecula, CA). Monoclonal anti-β₁-integrin (I41720) antibody was obtained from Transduction (Heidelberg, Germany). Monoclonal anti-vinculin (V9131) was obtained from Sigma-Aldrich (Munich, Germany). Monoclonal anti-matrix metalloproteinase

(MMP)-1 (MAB900), anti-MMP-13 (MAB511) and polyclonal anti-active caspase-3 (AF835) antibodies were provided by R&D Systems, Inc., (Heidelberg, Germany). Culture medium containing 5% FCS, 25 µg/mL ascorbic acid, 50 IU/mL streptomycin, 50 IU/mL penicillin, 2.5 µg/mL amphotericin B, 1% glutamine and 1% essential amino acids was obtained from Seromed (Munich, Germany). O.C.T. compound embedding medium and trypsin/ethylene diamine tetra-acetic acid (EDTA) (EG 3.4.21.4) were purchased from Sigma-Aldrich. Epon was obtained from Plano (Marburg, Germany). The two quinolones were used as commercially available solutions for intravenous (i.v.) infusion [Ciprobay[®] 400 Infusionslösung (Bayer, Leverkusen, Germany) and Tavanic[®] i.v. 500 mg (Aventis, Frankfurt am Main, Germany)]. Dexamethasone was purchased from Sigma-Aldrich and a 10 mM stock solution was prepared in ethanol. First, the solutions with the highest concentration used here (10 mg/L quinolone and 10 µM dexamethasone) were prepared from the quinolone or glucocorticoid preparations plus culture medium. Then, the other solutions were made by further dilution with culture medium. For the incubation media containing quinolone plus dexamethasone, the glucocorticoid was added at the indicated concentration to quinolone-containing culture media.

2.3. Electron microscopy

To assess the formation of tenocytes and tenocyte extracellular matrix in the monolayer cultures after 4 days of incubation at the ultrastructural level, transmission electron microscopy (TEM) was performed as previously described in detail [24]. Briefly, cells were fixed in Karnovsky's solution, post-fixed in 1% OsO₄ and then rinsed and subsequently dehydrated in an ascending alcohol series and finally embedded in Epon. Sections of ca. 50–70 nm were cut on a Reichert-Jung Ultracut E

microtome (Heidelberg, Germany). The ultrathin sections were contrasted with 2% uranyl acetate/lead citrate. A transmission electron microscope (TEM 10; Zeiss, Jena, Germany) was used to examine the tenocytes cultures.

2.4. Western blot analysis

Western blot analysis was performed as described previously in detail [21]. Briefly, tenocyte proteins were extracted with lysis buffer [50 mM Tris/HCl (pH 7.2)], 150 mM NaCl, 1% (v/v) Triton X-100, 1 mM sodium orthovanadate, 50 mM sodium pyrophosphate, 100 mM sodium fluoride, 0.01% (v/v) aprotinin, 4 µg/mL pepstatin A, 10 µg/mL leupeptin and 1 mM PMSF for 30 min on ice. The total protein concentration was determined using the bicinchoninic acid system Uptima (Interchim, Montluçon, France) with bovine serum albumin as a standard. Adjusted to an equal total cell protein concentration, samples were separated using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (5%, 7.5%, 10% or 12% gels) under reducing conditions and proteins were transferred onto nitrocellulose membranes. Western blotting was performed with different primary antibodies and alkaline phosphatase-conjugated secondary antibodies. To reveal the specific antigen–antibody complexes, nitro blue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (*p*-toluidine salt; Pierce, Rockford, IL) were used as substrates for alkaline phosphatase. Immunoblots were semiquantitatively analysed using the computer program 'Quantity one' (Bio-Rad, Munich, Germany). Experiments were performed triplicate.

3. Results

3.1. Culture of human-derived tenocytes

Tenocytes were isolated by a non-enzymatic method via outgrowth out of a dissected finger tendon. After ca. 1 week, tenocytes continuously migrated from the explant, adhered to Petri dishes and proliferated. Cells were plated with 12 mL of cell culture medium in a T75 flask and incubated at 37 °C in 5% CO₂. These primary tenocytes showed a typical elongated cell shape with long cytoplasmic processes. Three to five days later, when the cell density had approached confluence, these cells were removed using 0.05% trypsin/1.0 mM EDTA and were passaged up to a maximum of ten times in monolayer culture to gain sufficient cells.

3.2. Ultrastructural changes in tenocytes

The effect of the glucocorticoid and/or quinolones on tenocytes was examined by TEM. Serum-starved primary isolated tenocytes were either exposed to glucocorticoid and/or a quinolone or were left unexposed. Untreated control monolayer tenocytes showed a fibroblast-like shape, numerous small ridge-like or cuspidal surface processes, a large mostly euchromatic nucleus with nucleoli and well structured cytoplasm organelles, a well developed rough endoplasmic reticulum (ER), mitochondria and a large Golgi apparatus from the beginning of cultivation onwards (Fig. 1A,B). They produced a thin, pericellular matrix rim consisting of fine irregularly running filaments that could be demonstrated extracellularly on the cell surface (inset Fig. 1A). By contrast, when the tenocytes were exposed to either ciprofloxacin (Fig. 1C), levofloxacin (Fig. 1D) or dexamethasone (Fig. 1E) they showed degenerative changes such as swelling of the rough ER and clustering of swollen mitochondria and degeneration of other cell organelles. These changes

included areas of condensed heterochromatin in the cell nuclei. The flattened monolayer tenocytes became more and more rounded, lost their microvilli-like processes and became apoptotic (Fig. 1C–E). Both quinolones caused alterations at concentrations that are usually achieved under therapeutic conditions (3–10 mg/L). Dexamethasone-exposed tenocytes showed the abovementioned cellular alterations even at the lowest concentration tested (0.1 nM).

Tenocytes that were exposed to the combination of 3 mg/L quinolone plus 0.1 nM dexamethasone showed more pronounced degenerative changes such as multiple vacuoles, swelling of the rough ER, clustering of swollen mitochondria and Golgi apparatus (Fig. 1F,G) as well as degeneration of other cell organelles compared with those after treatment with a single drug alone.

3.3. Biochemical changes in the extracellular matrix and cytoskeleton

Western blotting was used to analyse biochemical changes at the protein level of tenocytes. The highest dexamethasone concentration tested (10 μ M) decreased collagen type I by 35%. Effects with ciprofloxacin at both concentrations (3 mg/L and 10 mg/L) and a combination of both drugs at low concentrations caused a similar decrease. A synergistic effect of 10 mg/L ciprofloxacin plus 1 nM dexamethasone was observed. Collagen type I production (Fig. 2) and synthesis of the β_1 -integrin (Fig. 3) were drastically reduced.

Comparison between the collagen and β_1 -integrin data showed that the β_1 -integrins reacted more sensitively than the matrix protein. Therapeutically relevant low concentrations of 0.1 nM dexamethasone did not induce such changes, but at the

higher concentration of 1 nM dexamethasone the variability was high, since some, but not all, samples showed changes. Levofloxacin in combination with dexamethasone caused similar changes, but only at concentrations of ≥ 10 mg/L levofloxacin (data not shown).

The mean amount of the cytoskeleton protein vinculin was suppressed by the two higher concentrations of dexamethasone to 62% (0.1 μ M) and 20% (10 μ M) of the dexamethasone mean control value, whereas no changes were observed for 0.1 nM and 1 nM compared with the untreated control (Fig. 4). Moreover, incubation with ciprofloxacin alone resulted in a strong decrease of vinculin, which was more pronounced than after incubation of the tenocytes with levofloxacin (data not shown).

All changes in protein concentrations occurred time dependently over the study period of 4 days, and with a combined exposure of dexamethasone plus a quinolone the changes were detectable at an earlier time point during the 4-day period (time dependency not shown).

3.4. Alterations in the amount of matrix metalloproteinases

The effects of levofloxacin and ciprofloxacin alone or in combination with dexamethasone on MMP-1 and MMP-13 were very pronounced. As described previously, densitometric analysis revealed an increase in MMP-13 of ca. 7-fold or 18-fold compared with controls following incubation for 4 days with 3 mg/L or 10 mg/L ciprofloxacin, respectively [25]. Incubation with 0.1 μ M or 10 μ M dexamethasone also evoked a clear-cut upregulation of MMP-1 and MMP-13 (Figs 5 and 6).

Following exposure of the cells to a combination of a quinolone plus dexamethasone, changes occurred more rapidly than after exposure to a quinolone alone and were even more pronounced. After 3 days in culture with 10 mg/L ciprofloxacin or levofloxacin plus 1 nM dexamethasone the increase in MMP-13 was approximately twice as pronounced in comparison with the effects with dexamethasone alone. After 4 days in culture, the amount of MMP-13 increased significantly with either 10 mg/L ciprofloxacin or levofloxacin plus 1 nM dexamethasone (Figs 6–8).

3.5. Activation of caspase-3

The amount of the apoptosis marker activated caspase-3 was very low in control cells (0–0.03 densitometric units). No significant change was observed after incubation with 0.1 nM or 1 nM dexamethasone; however, it was increased by the two higher concentrations of the glucocorticoid (Fig. 9). With 10 μ M dexamethasone the effect was noticed 1 day earlier compared with the 0.1 μ M concentration (data not shown).

A time- and concentration-dependent increase in caspase-3 was observed following incubation with the quinolones [25]. In comparison with incubation with 0.1 nM dexamethasone alone, combined exposure of the cells to 3 mg/L of either ciprofloxacin or levofloxacin plus 0.1 nM dexamethasone increased the concentration of caspase-3 from Day 3 onwards (Fig. 8B). Similar effects occurred following incubation with 10 mg/L quinolone plus 1.0 nM dexamethasone. However, for 10 mg/L ciprofloxacin plus 1.0 nM dexamethasone the changes were less pronounced than for 3 mg/L ciprofloxacin plus 0.1 nM dexamethasone. The reason for this unexpected finding is unknown.

4. Discussion

In a previous publication we have described that the quinolones ciprofloxacin and levofloxacin caused a time- and concentration-dependent decline in extracellular matrix and key signalling proteins in human tenocytes in vitro [25]. Furthermore, an increase in the apoptosis marker activated caspase-3 as well as characteristic ultrastructural degenerative features were observed in the quinolone-treated tenocytes. These alterations occurred at concentrations that are readily achieved in plasma and tissues of patients during therapy with these quinolones. In this continuative study, the effects of the glucocorticoid dexamethasone either alone or in combination with one of the quinolones ciprofloxacin or levofloxacin were analysed in our in vitro tenocyte model to study a possible synergism between glucocorticoids and quinolones. Dexamethasone was also studied at therapeutically relevant concentrations. A mean peak plasma concentration (C_{max}) of $8.4 \pm 3.6 \mu\text{g/L}$ (ca. $2 \cdot 10^4$ nM) has been reported following an oral dose of 1 mg dexamethasone [31–34].

The effects of dexamethasone alone were less distinct than those observed with quinolones and occurred only at the two higher concentrations tested. However, by combining ciprofloxacin or levofloxacin with dexamethasone at therapeutically relevant concentrations, synergistic effects on tenocytes were obvious for most of the analysed proteins compared with the effects observed with either drug alone. Importantly, during the culture period of 4 days most of the effects occurred earlier with a drug combination than with exposure to one drug alone. This supports the clinical observations of an increased risk for quinolone-induced tendinopathy by concomitant use of glucocorticoids [7].

Whereas the quinolone-induced tendon disorders are widely accepted as typical adverse effects of these drugs, the association of glucocorticoid use with a tendinopathy is discussed controversially. In vitro or in vivo data of the effects of glucocorticoids on tendon or cartilage are scarce. The observed decline in collagen type I following incubation with 10 μ M dexamethasone in our experiments is in agreement with the findings of Wong et al. [27]. In their in vitro model with human tenocytes, 1 μ M dexamethasone led to a decrease in cell proliferation, viability and collagen type I production [27]. Moreover, reduced expression of collagen type I mRNA and procollagen type I and II was shown in two studies with tenocytes of chick embryos [35,36]. A concentration-dependent reduction in the number of tenocytes and collagen synthesis was also detected with rat tail tenocytes when exposed over extended time periods [37].

All data indicate a direct effect of dexamethasone on the proliferation of tendon cells. Our finding of an increased amount of MMP-1 and MMP-13 following incubation with 0.1 μ M and 10 μ M dexamethasone indicates an additional catabolic effect on the extracellular tendon matrix. MMP-1 and MMP-13 are able to cleave collagen type I [38–40]. Few data on the glucocorticoid-mediated regulation of MMP production in tendons are available. Dexamethasone inhibited the upregulation of both MMP-2 and MMP-9 induced by the attachment of tendon cells to collagen type I [41]. In chondrocytes, reduced MMP production after incubation with dexamethasone was also detected, but in these experiments cells were pre-stimulated with cytokines to induce MMP production [42–44]. Obviously the effect of glucocorticoids on MMP expression differs depending on MMP species (collagenases versus gelatinases), cytokine pre-treatment and various other conditions.

The decreased amount of β_1 -integrin receptors in tenocytes following dexamethasone exposure can be interpreted as a sign of a slight impact on the initial step of signalling pathways such as the MAP kinase pathway as well as on cell–cell and cell–matrix interactions. Higher concentrations of dexamethasone induced apoptosis as indicated by increased caspase-3 activity as a downstream effector caspase and by pro-apoptotic ultrastructural changes. Wong et al. [27] did not find an indication for programmed cell death following dexamethasone incubation in their tenocyte in vitro model, which might be explained by the lower concentrations tested and the other endpoints used. Other publications describe that glucocorticoids induce apoptosis in human [45], rodent [46] or bovine [47] chondrocytes.

The mechanism of the synergistic effect between glucocorticoids and quinolones is still unknown. Data from in vivo and in vitro studies or clinical trials are scarce. An in vitro study with triamcinolone acetonide (100 $\mu\text{g}/\text{mL}$) combined with ciprofloxacin (100 $\mu\text{g}/\text{mL}$), pefloxacin or sparfloxacin showed enhanced cytotoxicity (mitochondrial dehydrogenase activity; MTT test) in comparison with incubation with the quinolone alone [48]. However, triamcinolone acetonide alone was more cytotoxic than ciprofloxacin in this model [48]. The origin of the human tendon cells (Achilles tendon versus finger tendon cells), the details of the experimental conditions and the respective glucocorticoid differ from our experiments.

In rats, dexamethasone inhibited pefloxacin-induced changes in Achilles tendons [49]. This is in contrast to clinical observations and to our results but might be explained by the short treatment period of only 24 h. In a typical clinical situation,

patients are treated chronically with glucocorticoids for indications such as pulmonary diseases or following organ transplantation [14,50].

Taken together, the effects of quinolones on human tenocytes were significantly intensified by dexamethasone depending on the quinolone, the concentration used and the duration of exposure. Our data support the clinical observations that treatment with glucocorticoids has to be considered an important risk factor for quinolone-induced tendinopathy.

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Competing interests

JS is currently employed at Bayer-Schering Pharma AG. HL is a consultant/speaker for Astellas Pharma, Bayer-Schering Pharma, Sanofi-aventis, Janssen, Pfizer and Wyeth. RS has received lecture fees from MSD. MS and MS-K declare no competing interests.

Ethical approval

Not required.

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Fig. 1. Transmission electron microscopy images of tenocytes exposed to a quinolone and/or dexamethasone as well as untreated tenocytes after a 4-day culture period in monolayer culture. (A,B) Untreated monolayer tenocytes (controls) showed a typical flattened shape with small cytoplasmic processes, a large mostly euchromatic nucleus with nucleoli, and well structured cytoplasm organelles such as mitochondria (arrowheads), rough endoplasmic reticulum (ER) (thin black arrows) and Golgi apparatus (white arrows). Inset (A): Synthetic activity is shown by formation of a thin matrix rim on the cell surface (star). (C–G) Exposure of tenocyte monolayer cultures to 3 mg/L ciprofloxacin (C), 3 mg/L levofloxacin (D), 0.1 nM dexamethasone (E), 3 mg/L ciprofloxacin plus 0.1 nM dexamethasone (F) and 3 mg/L levofloxacin plus 0.1 nM dexamethasone (G) led to degenerative changes such as multiple vacuoles, swelling of the rough ER (thin black arrows), clustering of swollen mitochondria (arrowheads) and degeneration of other cell organelles. The flattened monolayer tenocytes became more and more rounded, lost their microvilli-like processes and became apoptotic, including areas of condensed heterochromatin in the cell nuclei and multiple and autophagic cytoplasmic vacuoles (thick black arrows). Tenocytes treated with a quinolone and dexamethasone showed more severe cellular degeneration at the ultrastructural level (F,G). Magnification: (A,C–G) $\times 5000$, bar = 1 μM ; (B) $\times 10\,000$, bar = 2 μm ; (inset) $\times 10\,000$, bar = 2 μm .

Fig. 2. Immunoblot of collagen type I obtained with tenocytes after a culture period of 4 days and exposure to (A) 0.1 nM, 1 nM, 0.1 μM or 10 μM dexamethasone alone (green), (B) 3 mg/L ciprofloxacin plus 0.1 nM dexamethasone (blue) and (C) 10 mg/L ciprofloxacin plus 1 nM dexamethasone (blue). Data are presented as densitometric units (percent of controls) from each of three replicate experiments. To allow a direct comparison, data with ciprofloxacin alone (B and C, red), which have been published

previously, are also included [25]. A decrease in collagen type I was observed with the highest concentration of dexamethasone and all samples after exposure to ciprofloxacin. The most pronounced decrease in this matrix protein was observed after exposure to the high concentrations of ciprofloxacin plus dexamethasone (10 mg/L plus 1 nM).

Fig. 3. Immunoblot of β_1 -integrin obtained with tenocytes after a culture period of 4 days and exposure to (A) 0.1 nM, 1 nM, 0.1 μ M and 10 μ M dexamethasone alone (green), (B) 3 mg/L ciprofloxacin plus 0.1 nM dexamethasone (blue) and (C) 10 mg/L ciprofloxacin plus 1 nM dexamethasone (blue). Data are presented as densitometric units (percent of controls) from each of three replicate experiments. To allow a direct comparison, data with ciprofloxacin alone (B and C, red), which have been published previously, are also included [25]. A decrease in β_1 -integrin was observed with the two highest concentrations of dexamethasone and all samples after exposure to ciprofloxacin. The decrease was more pronounced after combined exposure in comparison with exposure to dexamethasone or ciprofloxacin alone.

Fig. 4. Immunoblot of vinculin obtained with tenocytes after a culture period of 4 days and exposure to (A) 0.1 nM, 1 nM, 0.1 μ M and 10 μ M dexamethasone (green), (B) 3 mg/L ciprofloxacin plus 0.1 nM dexamethasone (blue) and (C) 10 mg/L ciprofloxacin plus 1 nM dexamethasone (blue). Data are presented as densitometric units (percent of controls) from each of three replicate experiments. To allow a direct comparison, data with ciprofloxacin alone (B and C, red), which have been published previously, are also included [25]. A decrease in vinculin was observed with the two highest concentrations of dexamethasone and all samples after exposure to ciprofloxacin. Overall, a similar pattern of changes was observed as obtained with collagen type I.

Fig. 5. Immunoblot of matrix metalloproteinase-1 (MMP-1) obtained with tenocytes after a culture period of 4 days and exposure to (A) 0.1 nM, 1 nM, 0.1 μ M and 10 μ M dexamethasone alone (green), (B) 3 mg/L ciprofloxacin plus 0.1 nM dexamethasone (blue) and (C) 10 mg/L ciprofloxacin plus 1 nM dexamethasone (blue). Data are presented as densitometric units from each of three replicate experiments; the range of all controls (0.01–0.12 densitometric units) is presented as a shaded area. To allow a direct comparison, data with ciprofloxacin alone (B and C, red), which have been published previously, are also included [25]. A clear-cut increase in MMP-1 was observed with the two highest concentrations of dexamethasone; moreover, after exposure to 10 mg/L ciprofloxacin plus 1 nM dexamethasone all three values are above the control range.

Fig. 6. Immunoblot of matrix metalloproteinase-13 (MMP-13) obtained with tenocytes after a culture period of 4 days and exposure to (A) 0.1 nM, 1 nM, 0.1 μ M and 10 μ M dexamethasone alone (green), (B) 3 mg/L ciprofloxacin plus 0.1 nM dexamethasone (blue) and (C) 10 mg/L ciprofloxacin plus 1 nM dexamethasone (blue). Data are presented as densitometric units from each of three replicate experiments; the range of all controls (0.0–0.12 densitometric units) is presented as a shaded area. To allow a direct comparison, data with ciprofloxacin alone (B and C, red), which have been published previously, are also included [25]. A significant increase in MMP-13 was only observed with the highest concentration of dexamethasone. This protein was strongly increased after exposure to 10 mg/L ciprofloxacin plus 1 nM dexamethasone.

Fig. 7. Western blot of matrix metalloproteinase-13 (MMP-13) of human tenocytes after 1, 2, 3 or 4 days in culture. Cells were exposed to ciprofloxacin or levofloxacin at a concentration of 10 mg/L in combination with 1 nM dexamethasone. A clear-cut time dependency of the effect was noticed. Changes were more pronounced with ciprofloxacin than with levofloxacin.

Fig. 8. Time dependency of the effects of dexamethasone alone and in combination with ciprofloxacin or levofloxacin on (A) matrix metalloproteinase-13 (MMP-13) and (B) caspase-3. Columns represent results of Western blot analysis (mean values of densitometric units; $n = 3$). Ranges of control values were 10.0–63.3 densitometric units (MMP-13) and 6.7–20.0 densitometric units (caspase-3). Tenocytes were analysed after a culture period of 1, 2, 3 or 4 days. For MMP-13, the combination of the quinolones with dexamethasone at the higher concentrations (10 mg/L plus 1.0 nM, respectively) induced a clear-cut time-dependent effect that was not observed with dexamethasone alone. Similar effects were observed for caspase-3 at both concentration levels (3 mg/L plus 0.1 nM and 10 mg/L plus 1.0 nM).

Fig. 9. Immunoblot of caspase-3 obtained with tenocytes after a culture period of 4 days and exposure to (A) 0.1 nM, 1 nM, 0.1 μ M and 10 μ M dexamethasone alone (green), (B) 3 mg/L ciprofloxacin plus 0.1 nM dexamethasone (blue) and (C) 10 mg/L ciprofloxacin plus 1 nM dexamethasone (blue). Data are presented as densitometric units from each of three replicate experiments; the range of all controls (0.0–0.04 densitometric units) is presented as a shaded area. To allow a direct comparison, data with ciprofloxacin alone (B and C, red), which have been published previously, are also included [25]. An increase in caspase-3 was observed with the two highest concentrations of dexamethasone as well as after exposure to ciprofloxacin plus

dexamethasone at both concentration levels. However, for 10 mg/L ciprofloxacin plus 1.0 nM dexamethasone, changes were less pronounced than for 3 mg/L ciprofloxacin plus 0.1 nM dexamethasone.

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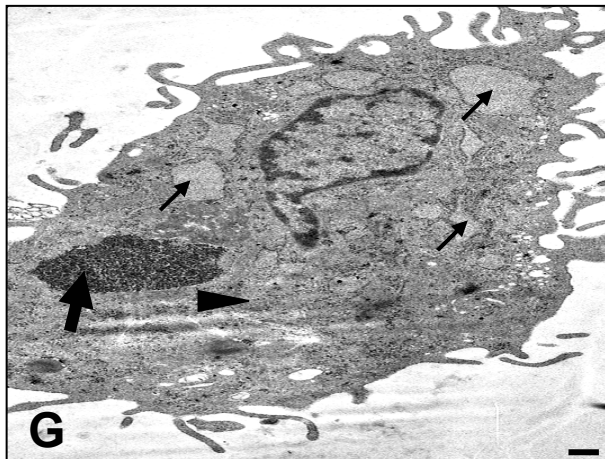
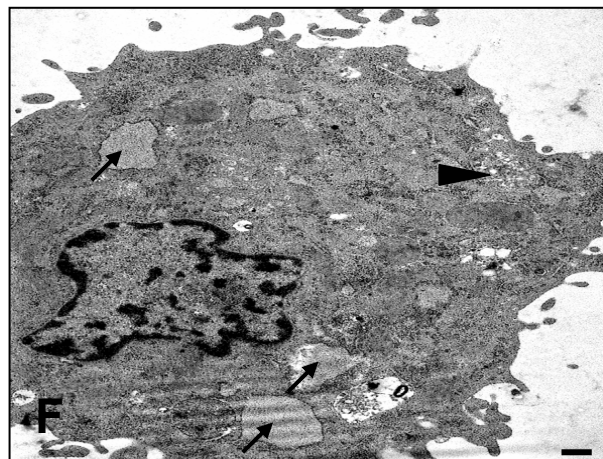
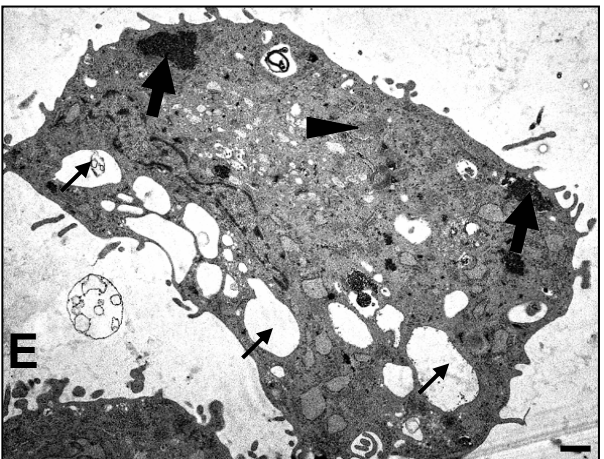
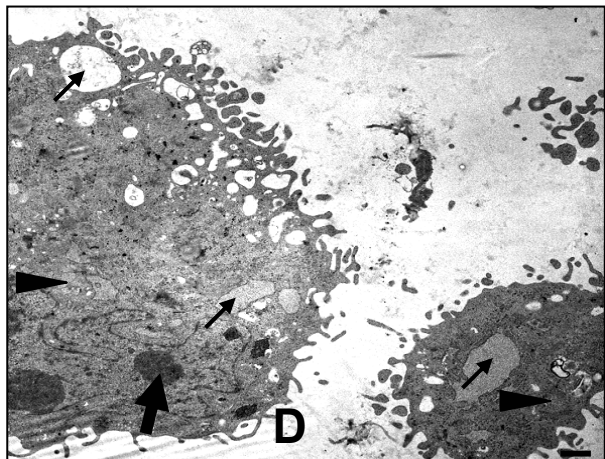
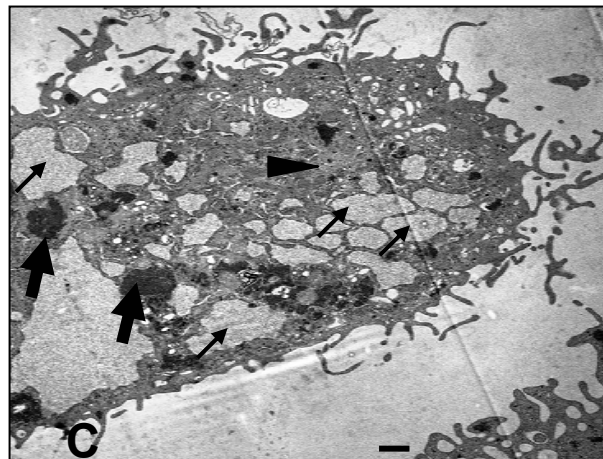
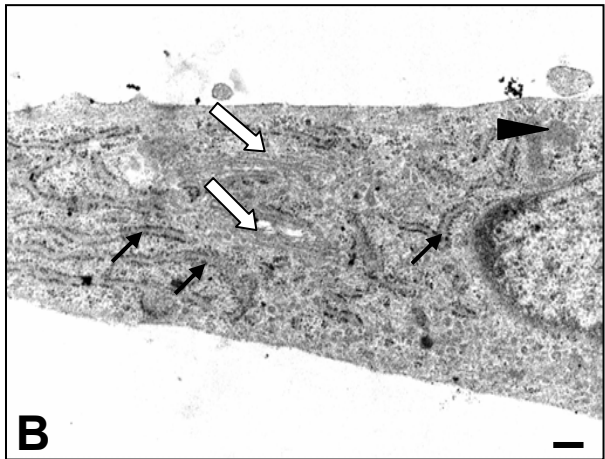
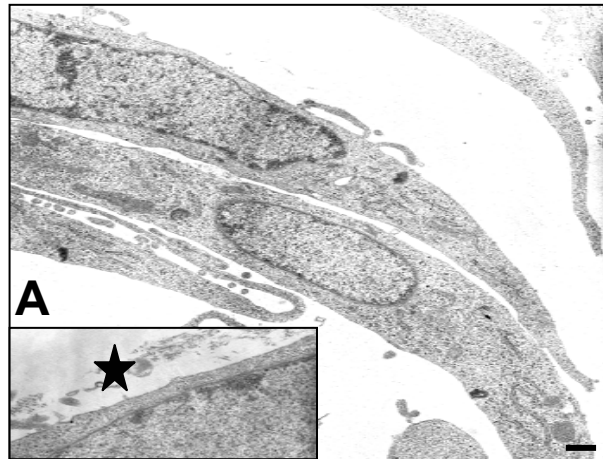
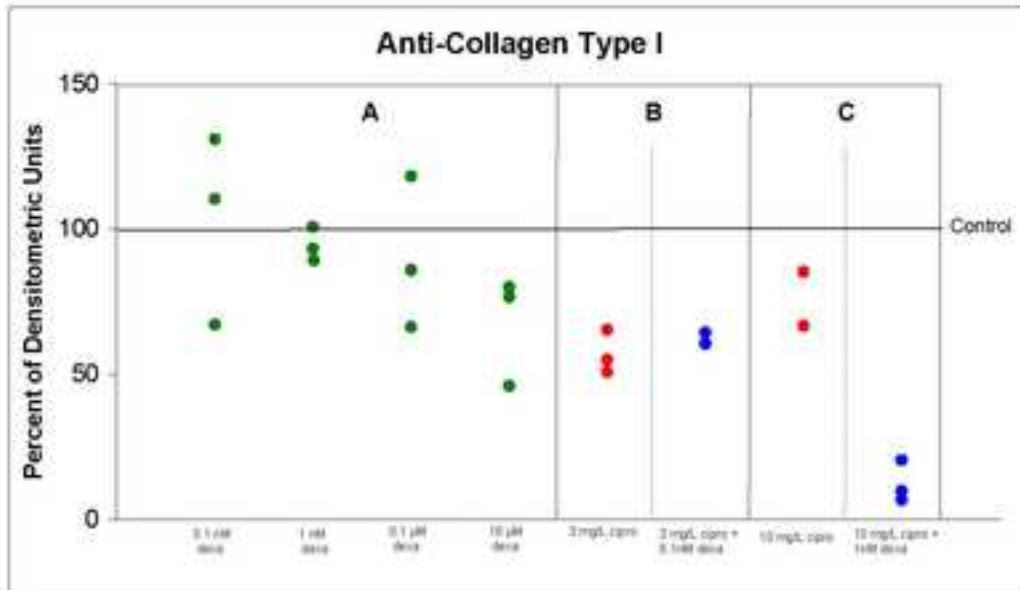
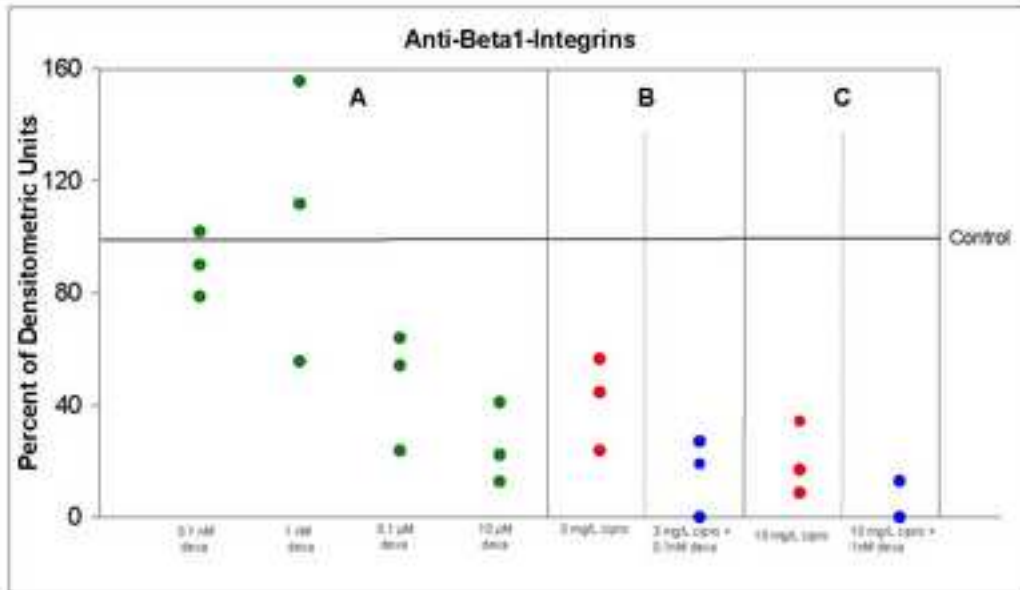
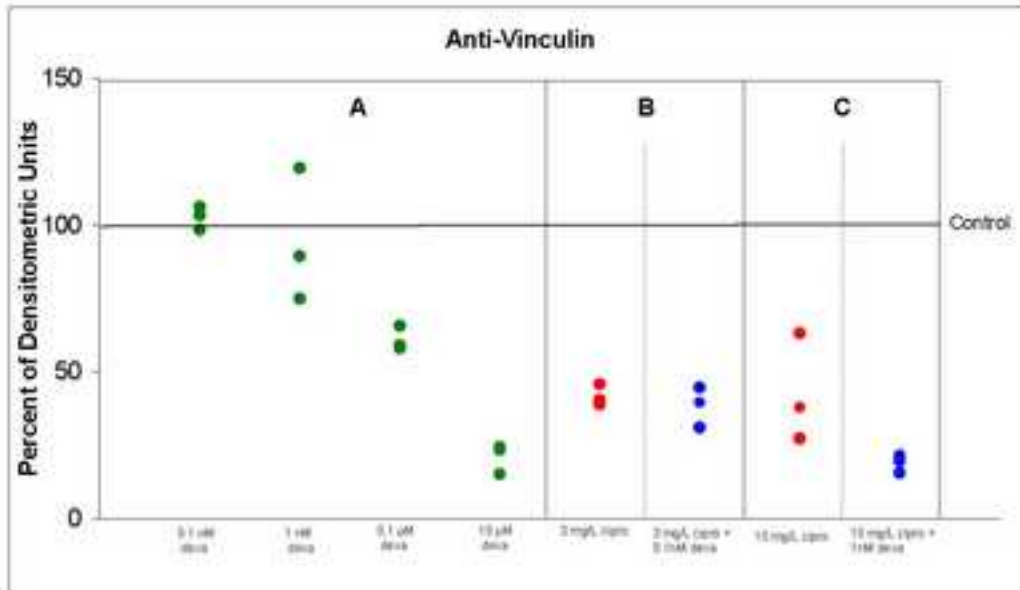
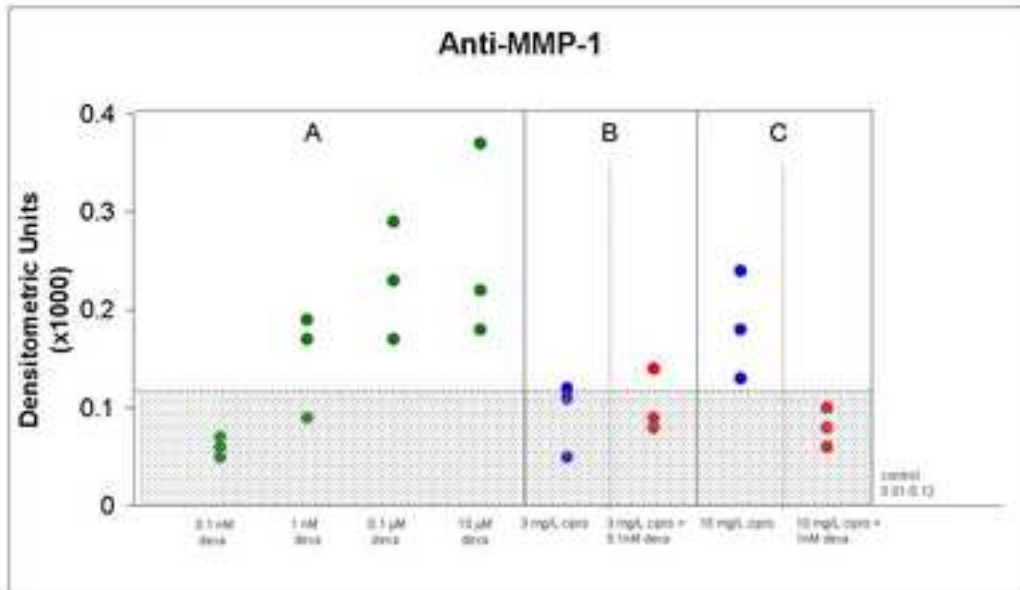


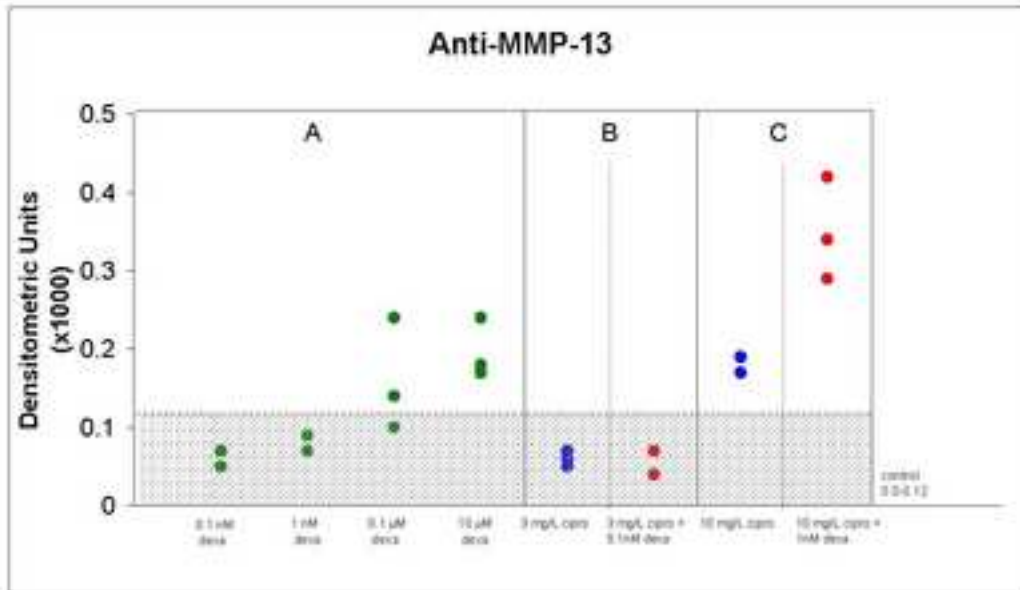
Fig. 1



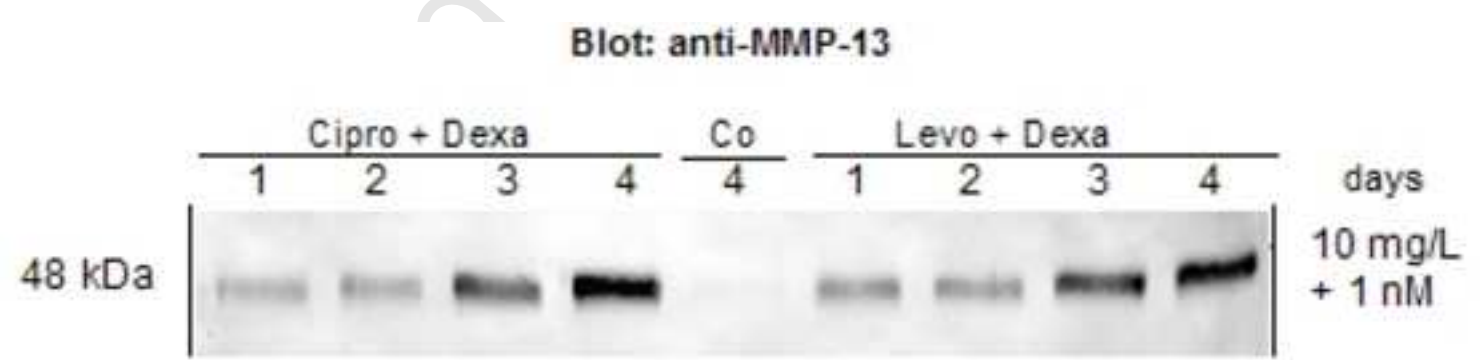




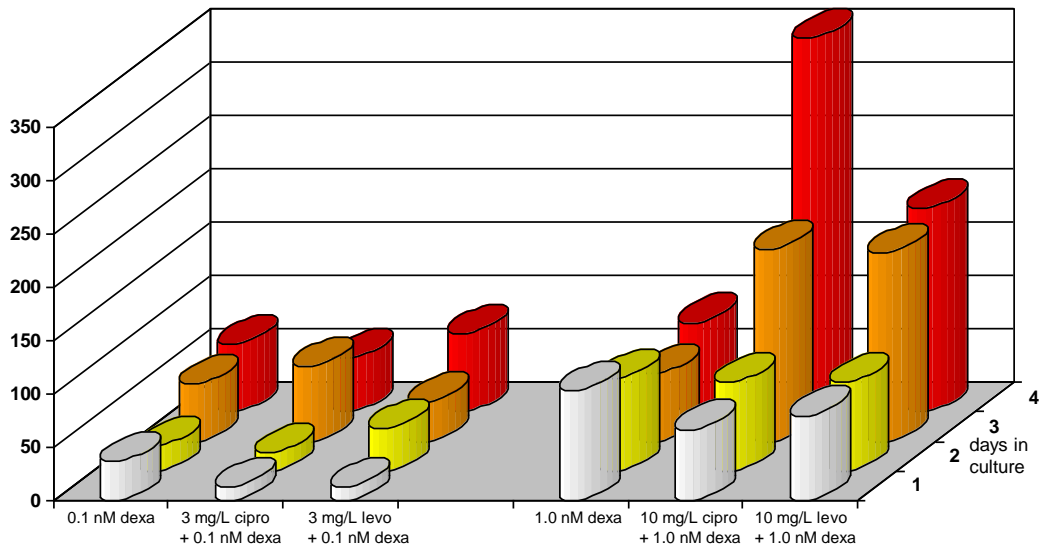




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