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Ciprofloxacin reduces the stimulation of prostaglandin E_2 output by interleukin-1β in human tendon-derived cells

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***Objective*. Fluoroquinolone antibiotics such as ciprofloxacin can induce tendon pathology and have various effects on tendon-derived cells in culture. We are investigating whether ciprofloxacin modifies signalling responses in tendon cells.**
***Methods*. Human Achilles tendon-derived cells were preincubated with or without ciprofloxacin (50 μg/ml) and were then challenged with interleukin-1β (IL-1β, 1 ng/ml) for up to 48 h. Prostaglandin E_2 (PGE_2) output was assayed by ELISA. The expression of cyclooxygenase-2 (COX-2) was examined by Western blotting.**
***Results*. IL-1β stimulated a substantial and prolonged increase in the output of PGE_2. Preincubation with ciprofloxacin reduced IL-1β-induced PGE_2 output at all times tested; the reduction at 48 h was 69% (99% confidence interval 59–79%; 15 experiments). Norfloxacin and ofloxacin also reduced PGE_2 output. However, ciprofloxacin did not affect the induction of COX-2 by IL-1β, measured at 4 or 48 h.**
***Conclusions*. Ciprofloxacin reduces IL-1β-induced PGE_2 output in tendon-derived cells. The reduction in PGE_2 output could modulate various cellular activities of IL-1β, and may be implicated in fluoroquinolone-induced tendinopathy.**

KEY WORDS: Tendon, Fluoroquinolone, Prostaglandin, Interleukin, Cyclooxygenase-2.

The fluoroquinolone antibiotics (ciprofloxacin, norfloxacin, ofloxacin, pefloxacin and others) have been used to treat a wide range of infections. Side-effects have been reported in the gastrointestinal tract and central nervous system [1], and additionally in cartilage and tendon [1, 2]. Tendon pain and/or rupture has been reported in a small proportion of patients in a number of clinical studies, Achilles tendons being the principal tendons affected [2]. Inflammation of the paratenon, disorganization of the extracellular matrix and degenerative changes in tendon cells have been noted in studies of fluoroquinolone-treated animals [3–5].

While the principal target of fluoroquinolone antibacterial activity is the enzyme DNA gyrase [6], several effects of fluoroquinolones on mammalian tissues and cells have been reported. Mitochondrial structure and function may be compromised [5, 7–10], which may underlie the cytotoxic and/or apoptotic effects occurring in various cell types treated with high concentrations of fluoroquinolones [8–10]. A non-cytotoxic reduction of tendon cell proliferation has also been reported [11]. Fluoroquinolones may modulate the expression of inflammatory cytokines [12–15], some of these effects being correlated with changes in transcription factor induction [13, 15]. Changes in the expression of extracellular matrix proteins have also been observed in organ- and cell-culture systems derived from cartilage and tendon [7, 11, 16], and the expression of proteinases was shown to be modulated by ciprofloxacin in canine [11] and human tendon-derived cells [17].

We found that ciprofloxacin potentiated IL-1β-stimulated expression of matrix metalloproteinase (MMP)-3 (mRNA and secreted proenzyme) in human Achilles tendon-derived cells [17]. Increased expression of MMP-1 mRNA was also observed [17]. These results prompted us to investigate whether ciprofloxacin modulates IL-1β-induced signalling responses in tendon-derived cells.

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Materials and methods

Materials

Ciprofloxacin was obtained from ICN (Basingstoke, UK), and was freshly dissolved at 10 mg/ml in 0.1 M HCl, except for some early experiments in which ciprofloxacin was dissolved in DMSO (as described in [17]). IL-1β was a gift from Glaxo Wellcome (Stevenage, UK) and aliquots (1 μg/ml) were stored at –70°C. Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS) and antibiotics were obtained from Invitrogen (Paisley, UK). Primary antibodies against β-actin and cyclooxygenase-2 (COX-2) were from Sigma (Poole, UK) and Santa Cruz (Insight Biotechnology, Wembley, UK) respectively. Secondary antibodies and detection reagent (CDP-Star) were by Dako (Ely, UK) and Tropix (Roche Diagnostics, Lewes, UK).

Cell isolation and incubation

Tendon specimens were obtained from tissue discarded during surgery for chronic Achilles tendinopathy, with informed patient consent and local ethical committee approval. Cells isolated by outgrowth from separate tendon explants were maintained and passaged in DMEM containing 10% (v/v) FCS, penicillin, streptomycin and 25 mM HEPES, and were used between passages 4 and 10. Cells were seeded at 10^5 cells/well in six-well plates and were incubated for 3 days before the experiment. They were rinsed with 2 ml of medium containing 10% FCS or in serum-free medium containing insulin, transferrin and selenium, and were then given 2 ml of the same medium with or without ciprofloxacin (50 μg/ml). After 48 h, the cells were again rinsed and given fresh medium containing ciprofloxacin and/or IL-1β (1 ng/ml). Control cultures had equivalent additions of HCl or DMSO as appropriate. After further incubation up to 48 h, the supernatant medium was removed and stored at –20°C, and the cells were extracted for Western blotting.

Western blotting

Cells were rinsed with ice-cold balanced salts solution, and were lysed for 15 min on ice using 300 μl of extraction buffer [10 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 50 mM NaF, 20 mM $Na_4P_2O_7$, 1 mM PMSF (phenylmethylsulphonyl fluoride), 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1% NP-40]. The extracts were centrifuged to remove debris (2 min at 12 000 *g* at 4°C) and were stored at –70°C. Aliquots (20 μl) were subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis under reducing conditions, using a 10% (w/v) polyacrylamide resolving gel with a 4.5% (w/v) stacking gel. The proteins were electroblotted onto PVDF (polyvinylidene fluoride) membranes. These were blocked, incubated with primary antibodies, washed, incubated with secondary antibodies, washed and developed using standard protocols.

Prostaglandin E_2 assay

Samples of cell supernatants were assayed in duplicate using a colorimetric competitive enzyme-linked immunosorbent assay (ELISA) for prostaglandin (PG) E_2 (R&D Systems, Abingdon, UK) according to the supplier's instructions. All dilutions of standards and samples were performed using culture medium. The effective range of the assay was 39–5000 pg/ml. For the analysis of early time-points, a high-sensitivity PGE_2 ELISA from the same supplier was used: the effective range of this assay was 8–1000 pg/ml. The addition of ciprofloxacin, DMSO or IL-1β at concentrations equivalent to those present in the cell supernatants did not affect the values obtained for PGE_2 standards.

Replication and presentation of data

The principal effect observed (the decrease in IL-1β-stimulated PGE_2 output) was obtained in at least two experiments with cells isolated from each of five separate tendons. Because PGE_2 output varied between experiments, the results in each experiment were generally normalized to the value for cells treated with IL-1β after control preincubation, defined as 100%. Significant differences from the 100% value were determined from the 95 or 99% confidence intervals (CI) of the different treatments ($P < 0.05$ and $P < 0.01$ respectively).

Results

The basal output of PGE_2 by tendon-derived cells was close to or below the minimum detection limit of the assay (40 pg/ml, corresponding to about 80 pg/10^5 cells in 48 h). In serum-free medium, PGE_2 output from IL-1β-stimulated cells remained close to the minimum detection limit (the maximum observed being 135 pg/ml in 48 h, in one of five separate experiments) and it was not possible to deduce a consistent pattern of effect of ciprofloxacin (data not shown). In contrast, when cells were incubated in medium containing 10% FCS, IL-1β stimulated a substantial, rapid and prolonged increase in PGE_2 output (Fig. 1). There was considerable variation between experiments in the magnitude of IL-1β-stimulated PGE_2 output, ranging between 1.2 and 65 ng/ml after 48 h (Fig. 1A; mean $\pm$ S.E.M., 19 ± 5 ng/ml; $n = 15$). Irrespective of the magnitude of stimulation, pretreatment of the cells with ciprofloxacin reduced PGE_2 output in IL-1β-stimulated cells (Fig. 1A). The reduction was 69% at 48 h compared with cells given control pretreatment (99% CI 59–79; $n = 15$), and was evident from early time-points (Fig. 1B), being 82% after 4 h and 68% after 8 h (95% CI 64–100 and 40–96 respectively; $n = 3$). Pretreatment with the related fluoroquinolones norfloxacin and ofloxacin also reduced the stimulation of PGE_2 output, by 55 and 46% over 48 h (99% CI 26–82 and 19–71 respectively; $n = 3$), but the non-fluorinated quinolone nalidixic acid was more variable in effect, giving 77% inhibition in one of three experiments but no significant effect overall (data not shown).

Because the stimulated production of PGE_2 frequently involves increased expression of the PGH synthase enzyme COX-2 [18], we examined the levels of COX-2 in tendon cells stimulated with IL-1β after pretreatment with and without ciprofloxacin. Control cells showed little or no immunodetectable COX-2 protein (Fig. 2). Substantial induction of COX-2 expression was stimulated by IL-1β within 4 h and was maintained up to 48 h (Fig. 2). Ciprofloxacin did not affect either the basal or the IL-1β-stimulated expression of COX-2 at 4 or 48 h (Fig. 2).

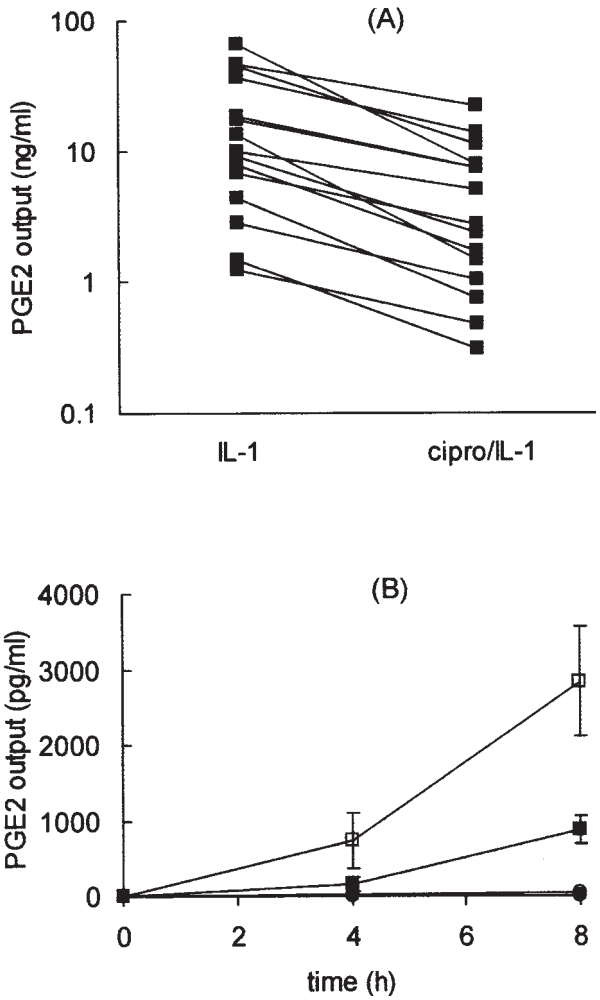

FIG. 1. PGE_2 output by human tendon-derived cells. Cells were pretreated for 48 h with or without ciprofloxacin, and were then incubated with ciprofloxacin (cip) and/or IL-1β for between 4 and 48 h. Cell supernatants were analysed by ELISA for PGE_2. (A) PGE_2 output over 48 h by IL-1β-treated cells after control pretreatment or pretreatment with ciprofloxacin. The output from cells incubated without IL-1β was less than 0.1 ng/ml, and is not shown. Results from 15 separate experiments. (B) Early time-course showing cells treated with IL-1β (squares) or without IL-1β (circles), after control pretreatment (open symbols) or pretreatment with ciprofloxacin (filled symbols). Mean $\pm$ S.E.M. from three experiments.

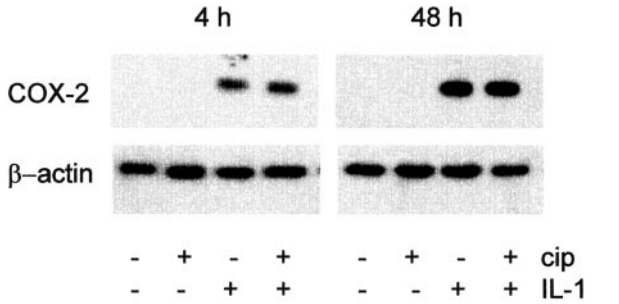

FIG. 2. Expression of cyclooxygenase-2 in human tendon-derived cells. Cells were pretreated for 48 h with or without ciprofloxacin, and were then incubated with ciprofloxacin (cip) and/or IL-1β for a further 4 or 48 h. Cell extracts were analysed by Western blotting using antibodies specific for COX-2 (upper panels) or β-actin (lower panels) as the loading control. Similar results were obtained in two additional experiments.

Discussion

Fluoroquinolones have been reported to have a range of effects on mammalian cells, both *in vivo* and *in vitro*, ranging from gross ultrastructural perturbation of organelles such as mitochondria to specific and contrasting changes in gene expression (see Introduction). We are investigating whether fluoroquinolones affect signalling responses in human tendon cells.

As in other cell-types [19, 20], IL-1β induced a prolonged increase in COX-2 expression and PGE_2 output in human tendon fibroblasts (Figs 1 and 2). Ciprofloxacin did not affect the expression of COX-2 after treatment with IL-1β (Fig. 2), or the IL-1β-induced phosphorylation of the signalling kinases p54 JNK/SAPK and p38MAPK (data not shown). These results established that preincubation with ciprofloxacin did not generally compromise or enhance signal transduction at the IL-1 receptor. However, ciprofloxacin (and other fluoroquinolones) reduced the output of PGE_2 induced by IL-1β in tendon cells by up to 90% (Fig. 1). This reduction in PGE_2 output was similar in magnitude to that induced by low concentrations of the COX-2 inhibitors indomethacin and NS398 (data not shown). It is possible that ciprofloxacin might also act as a COX-2 inhibitor; alternatively, the reduction in stimulated PGE_2 output could be due to reduced mobilization of arachidonic acid (the precursor for PG synthesis), by inhibition of phospholipase activation or activity [18]. We are not aware of any data currently available that would distinguish between these possibilities. In contrast to our data for PGE_2, an increase in PGI_2 was reported for endothelial cells treated with fluoroquinolones [9]. This may indicate either that the effect of fluoroquinolones on prostanoid synthesis is cell-type-specific or that it occurs downstream of COX-2 [18].

A reduction in PGE_2 output provides a mechanism by which fluoroquinolones might affect a variety of cellular responses. For example, the use of indomethacin to inhibit PG production, thereby reducing feedback inhibition, can enhance the IL-1-induced expression of matrix-related genes, including MMPs, in some cell types [21, 22]. A reduction in feedback inhibition, via reduced PGE_2 output, thus seemed to be a possible explanation for our previous observation that ciprofloxacin enhanced MMP expression induced by IL-1β in tendon cells [17]. However, we found that indomethacin and NS398 neither mimicked nor blocked the enhancement of MMP expression by ciprofloxacin in these cells (data not shown), indicating that additional mechanisms must underlie this effect.

The role of PGs in tendon physiology and pathology is uncertain. Although non-steroidal anti-inflammatory

drugs are commonly used in the treatment of tendinopathies, most histopathological studies have demonstrated the non-inflammatory (degenerative) nature of the condition, at least at the end stage of the disease [23, 24]. Furthermore, no significant increase in PG levels was detected in the peritendinous fluid in various tendinopathies [25, 26]. On the other hand, levels of PGE_2 were increased in the peritendinous space in the recovery period after strenuous exercise and there was evidence for increased collagen degradation [27]. Similarly, tendon cells exposed to cyclic strain *in vitro* showed increased expression of PGE_2, and increased DNA and protein synthesis, compared with unstretched control cells [28]. Indomethacin inhibited PGE_2 output and DNA synthesis in stretched cells, but increased levels of protein synthesis [29]. These data are consistent with the hypothesis that PGs are implicated in the adaptive response of the tendon, acting to modulate the remodelling of the extracellular matrix, and interference with this response could contribute to the tendinopathy induced by ciprofloxacin. However, the levels of PGs in ciprofloxacin-induced human tendinopathies have not yet been reported. It therefore remains to be determined whether a reduction in PG synthesis is one of the factors contributing to ciprofloxacin-induced tendinopathy.

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Conflict of interest

The authors declare that there is no conflict of interest with respect to the work described in this article.

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