

Leukotriene B₄ at low dosage negates the catabolic effect of prostaglandin E₂ in human patellar tendon fibroblasts

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Abstract

Tendinopathy often involves inflammation and matrix degeneration. The inflammatory mediators such as prostaglandin E₂ (PGE₂) and leukotriene B₄ (LTB₄) are implicated in the development of tendinopathy. Therefore, the purpose of this study was to determine the effect of PGE₂ and LTB₄ on the proliferation of human patellar tendon fibroblasts (HPTFs), the gene expression of collagen type I, MMP-1 and MMP-3, as well as the protein secretion of these gene products by the cells. The results showed that LTB₄ at low doses (0.1 and 1 nM) significantly increased cell proliferation compared to controls and LTB₄ at 0.1 nM negated the PGE₂-induced decrease in cell proliferation. In addition, PGE₂ at 100 ng/ml significantly increased the expression of MMP-1 and MMP-3 at both mRNA and protein levels. These stimulatory effects were significantly diminished by co-treatment with LTB₄ at 0.1 nM. Finally, neither PGE₂ nor LTB₄ treatment affected collagen type I gene expression. These results suggest that low levels of LTB₄ counterbalance the negative effects mediated by PGE₂ on tendon fibroblast proliferation and MMP production, which may lead to matrix degradation. Thus, our findings suggest that although LTB₄ is generally thought to be pathogenic, low levels of LTB₄ are actually beneficial in maintaining tendon tissue homeostasis.

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1. Introduction

Tendinopathy generally describes a group of tendon disorders marked by tendon inflammation and degeneration (Maffulli et al., 1998). Tendon inflammation is marked by the presence of inflammatory mediators, including prostaglandin E₂ (PGE₂) and leukotriene B₄ (LTB₄) (Almekinders et al., 1993). Although the exact amounts of both PGE₂ and LTB₄ in inflamed

tendons are not known, their levels are markedly increased when tendons or tendon fibroblasts are subjected to repetitive mechanical loading conditions (Langberg et al., 1999; Wang et al., 2003; Li et al., 2004). These data suggest that PGE₂ and LTB₄ may play a role in tendon degeneration, the late stages of tendinopathy (Wang, 2005). Tendon degeneration refers to degenerative changes in tendon matrix and is carried out by matrix metalloproteinases (MMPs) (Riley, 2005a,b), which are a group of enzymes essential for matrix turnover (Birkedal-Hansen et al., 1993; Fingleton and Matrisian, 2001). MMPs are present in high concentrations in inflammatory regions (Birkedal-Hansen, 1993) and degrade specific connective tissue matrix components such as collagen. For example, MMP-1 can directly cleave native collagen type I (Pardo and Selman, 2005), which is the dominant form of collagen in tendons, while MMP-3 degrades aggrecan and collagen-associated small glycoproteins (Sterlicht and Werb, 2001).

Abbreviations: LTB₄, Leukotriene B₄; PGE₂, Prostaglandin E₂; MMP, Matrix Metalloproteinase; HPTF, Human Patellar Tendon Fibroblast; DMEM, Dulbecco's Modified Eagle Medium; PCR, Polymerase Chain Reaction; EtOH, Ethanol; PMS, Phenazine Methosulfate; GAPDH, Glyceraldehyde Phosphate Dehydrogenase; ELISA, Enzyme-linked Immunosorbent assay; PKC, Protein Kinases C; NF-κB, Nuclear Factor kappa-B.

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Although MMPs are clearly involved in the pathogenesis of tendinopathy, the detailed molecular mechanisms of tendinopathy remain elusive. *In vitro* studies have shown that repetitive mechanical loading of tendon fibroblasts induces the release of PGE₂ and LTB₄ (Almekinders et al., 1995; Li et al., 2004). Both are also produced in substantial amounts at the sites of inflammation (Henderson, 1994; Lawrence et al., 2002). PGE₂ and LTB₄ are inflammatory mediators and have been implicated in the pathogenesis of several inflammatory diseases such as tendinopathy and rheumatoid arthritis (Davidson et al., 1983; Almekinders et al., 1993; Trebino et al., 2003). It has also been reported that the levels of both mediators are elevated in cases of tissue injury and dysfunctional joints as well as in inflamed tendons (Moreland et al., 1989; Quinn and Bazan, 1990). PGE₂ is a potent inhibitor of type I collagen synthesis in several connective tissue cell types such as fibroblasts (Varga et al., 1987; Riquet et al., 2000). In addition, elevated PGE₂ levels were found in the human tendon after repetitive mechanical loading (Langberg et al., 1999). Recently, we have shown that exogenous addition of PGE₂ at a dose of 100 ng/ml decreases proliferation and collagen production in human patellar tendon fibroblasts (HPTFs) (Cilli et al., 2004). On the other hand, LTB₄ at picomolar concentrations has been shown to stimulate DNA synthesis in cultured human epidermal keratinocytes and to induce proliferation of arterial smooth muscle cells in primary culture (Kragballe et al., 1985; Palmberg et al., 1989). Also, leukotrienes at low concentrations (0.1 to 1 nM) stimulate collagen synthesis of lung fibroblasts (Phan et al., 1987, 1988). Thus, although LTB₄ has been implicated in the pathogenesis of various inflammatory diseases (Davidson et al., 1983; Sharon and Stenson, 1984; Konstan et al., 1993), its potential role in the tendon tissue homeostasis as well as pathogenesis remains to be explored.

Therefore, the purpose of this study was to determine the effect of LTB₄ as well as its interactive effect with PGE₂ on proliferation, gene expression and/or protein production of collagen type I and MMPs including MMP-1 and MMP-3 in HPTFs. Our results show that exogenous LTB₄ at picomolar concentrations (≤ 100 pM) negated the decreased cell proliferation and the increased MMP-1 and MMP-3 gene expression and protein production that were induced by PGE₂. However, higher concentrations of LTB₄ (>1 nM) in combination with PGE₂ further decreased cell proliferation. Our results highlight the important role of LTB₄ in tendon homeostasis as well as in the pathogenesis of tendinopathy.

2. Materials and methods

2.1. Cell culture

Tendon samples were obtained from fresh surgical wastes of normal tendon grafts used for the reconstruction of the anterior cruciate ligament. The protocol for obtaining tendon samples was approved by the University of Pittsburgh Institutional Review Board. HPTFs were isolated and maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS, penicillin (50 U/ml), and streptomycin (50 U/ml) as previously described (Wang et al., 2003). The fibroblasts were

subcultured for 4–6 passages and were used for the experiments. After these passages, tendon fibroblasts maintained their morphology and doubling time. Cells from at least two donors were used in the experiments.

2.2. PGE₂ and LTB₄ treatments

For the proliferation experiments, HPTFs were plated in 6-well plates to attain roughly 50% confluence, which was equivalent to 4×10^4 cells per well. The cells were incubated at 37°C in a humidified atmosphere of 5% CO₂ in growth medium to permit attachment and an even distribution. After 24 h, varying concentrations of LTB₄ (Cayman Chemicals, Ann Arbor, Michigan), ranging from 10 pM to 10 nM, were added to the wells. PGE₂ was added to the cell cultures at a dose of 100 ng/ml because our previous study showed that this PGE₂ dose decreases HPTF proliferation and collagen production (Cilli et al., 2004). Fibroblasts treated with EtOH (0.1%), which is the vehicle for PGE₂ or LTB₄, were used as the control groups. The cells treated with both agents received LTB₄ 30 min prior to the addition of PGE₂. All the cells in the experimental and control groups were incubated for an additional 72 h. To determine the number of viable cells in the cultures, an MTS assay was performed (see below).

For RNA and protein measurements, cells at near confluence (2×10^5 /ml) were plated in triplicate in 6-well plates and incubated. After 24 h, LTB₄ at 0.1 nM was added for 30 min. Then PGE₂ at 100 ng/ml was added to the wells. In separate experiments, PGE₂ at the doses of 100, 250, and 500 ng/ml alone were added to cell cultures to examine dose-dependent effect on collagen type I gene expression. EtOH (0.1%), which is the vehicle for PGE₂ or LTB₄, was added to some wells as the control groups. After an additional 48 h of incubation, the conditioned media were collected for MMP protein measurements. Using an RNeasy kit (Qiagen Inc., USA), total RNA was extracted from the cells for measuring gene expression.

2.3. Cell proliferation assay

This assay uses a soluble tetrazolium salt, MTS, and the electron coupling reagent, phenazine methosulfate (PMS), to measure the dehydrogenase enzyme activity in metabolically active cells. MTS is chemically reduced by cells to formazan, which is soluble in tissue culture media. MTS solutions were prepared according to the manufacturer's instructions (Promega Corporation, Madison, WI). Stock PMS (Sigma Chemicals, St. Louis) was dissolved in PBS at a concentration of 0.92 mg/ml. These solutions were stored in light-protected tubes at -20 °C. MTS and PMS reagents were mixed at a ratio of 20:1 (MTS:PMS) immediately prior to addition to cell culture at a ratio of 1:5 (reagent:cell culture medium). After incubation for 1 h in a humidified atmosphere at 37 °C and 5% CO₂, triplicate samples of 200 μ l culture medium were aliquoted into 96-well plates and the absorbance was measured at 492 nm using a microplate reader. The absorbance value represented the number of viable cells in each sample since the production of formazan is proportional to the number of viable cells (Malich et al., 1997).

Table 1
Primer sequences for RT-PCR

Gene, annealing temperature	Primer sequence, forward/reverse	PCR product size (bp)
MMP-1, 58 °C	5'-CAACT CTGGAGTAAT GTCACA-3' 5'-T ACATCAAAGC CCCAGATCA-3'	295
MMP-3, 55 °C	5'-TTT TGG CCA TCT CTT CCT TCA-3' 5'-TGT GGA TGC CTC TTG GGT ATC-3'	138
Collagen-I, 58 °C	5'-GGT TAC TAC TGG ATT GAC C-3' 5'-TTG CCA GTC TCC TCA TCC-3'	328
GAPDH, 58 °C	5'-AAATCCATGGCAC CGTCAAGGCT-3' 5'-CTCATGGTTCACACC CATGACGAA-3'	295

2.4. RT-PCR

Reverse transcription (RT) was carried out with 1 µg of total cellular RNA using the ThermoScript RT-PCR System (Invitrogen) for first strand cDNA synthesis in 20 µl of reaction volume. The sequences for the primers and annealing temperature optimized for each primer set are provided in Table 1. For all experiments, the conditions were determined to be in the linear range for the PCR amplification. Briefly, all samples were subjected to RT and subsequent amplification of the cDNA samples was performed by PCR at the same time. The cDNA samples were then assessed for GAPDH expression. The levels of GAPDH mRNA did not vary with time after the addition of LTB₄ and PGE₂ or their combination following 28 PCR cycles. Genomic DNA was included for the PCR to ensure that there was no genomic DNA contamination in the total RNA samples. The cDNA was amplified by PCR using 28 cycles at 95 °C for 30 s, 55–60 °C for 30 s, and 72 °C for 30 s in the presence of *Taq* polymerase (Invitrogen), 50 pmol of sense and antisense primers. PCR products were resolved on 1.5% agarose gels by electrophoresis and visualized by staining with ethidium bromide and UV transillumination. Integrated density values for the genes in question were normalized to the GAPDH values to yield a semi-quantitative assessment of gene expression levels.

2.5. Assay for MMP-1 and MMP-3 protein production

Pro-MMP-1 and total MMP-3 (pro- and active MMP-3) concentrations in medium were determined using sandwich ELISA kits specific to human MMP-1 and MMP-3, respectively (R&D Systems, Minneapolis, MN, USA). The concentrations of MMP-1 and MMP-3 were determined using a standard curve in the range of 0.16–10 ng/ml and normalized to the cell number.

2.6. Data analysis

Data were expressed as mean ± SD. The absorbance values obtained for cell proliferation data were normalized to the

control. For the analysis of RT-PCR gene expression levels, the density values of the bands were normalized to the GAPDH values to yield a semi-quantitative assessment of gene expression. The amounts of MMP-1 and MMP-3 proteins were normalized to the respective cell number.

For statistical analysis of cell proliferation and collagen production, one-way ANOVA with a post hoc Bonferroni's test was used to determine which two groups showed significant differences. A difference between two groups was considered statistically significant if the *p* value was less than 0.05.

3. Results

3.1. LTB₄ regulated tendon fibroblast proliferation at low concentrations (<1 nM)

The effect of LTB₄ at various concentrations on the proliferation of HPTFs was determined. At 0.01 nM, the fibroblast proliferation only slightly increased. At 0.1 nM, however, cell proliferation significantly increased by 14% compared to untreated fibroblasts (*p*=0.002). Also, at 1 nM of LTB₄, fibroblast proliferation maintained a significant 14% increase (*p*=0.001). However, higher concentrations of LTB₄ (>1 nM) did not induce any significant fibroblast proliferation (Fig. 1).

We further examined the effect of LTB₄ and its combinations with PGE₂ on fibroblast proliferation. At 100 ng/ml, PGE₂ significantly decreased cell proliferation by 11% (*p*=0.02). However, when fibroblasts were treated with PGE₂ and 0.1 nM LTB₄ in combination, the effect of PGE₂ on cell proliferation was negated and proliferation was increased close to the levels of control cells (Fig. 2). Cells which were treated with a higher concentration of LTB₄ (10 nM) alone appeared to have no influence on the cell proliferation (Figs. 1 and 3). When this high concentration of LTB₄ was added to the cells in combination with 100 ng/ml PGE₂, LTB₄ failed to reverse the anti-proliferative action mediated by PGE₂. In fact, their combination further decreased fibroblast proliferation (*p*=0.001) compared to the effect of PGE₂ alone (Fig. 3). It was noted that all doses of

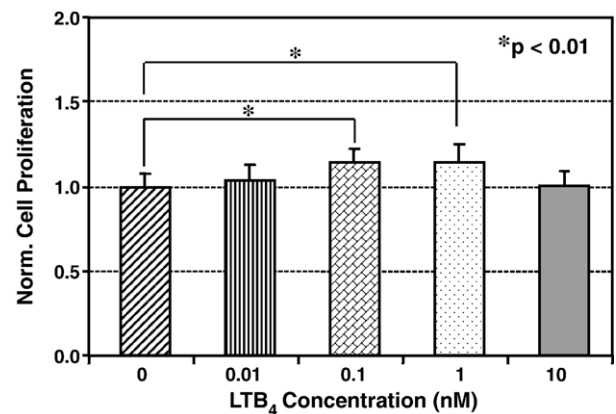


Fig. 1. The effect of LTB₄ on the proliferation of human patellar tendon fibroblasts. Low doses of LTB₄ (0.1 and 1 nM) significantly increased fibroblast proliferation, but a higher dose (10 nM) did not have any effect. Six independent experiments were performed, with a total sample size ranging from 8 to 18 for each group.

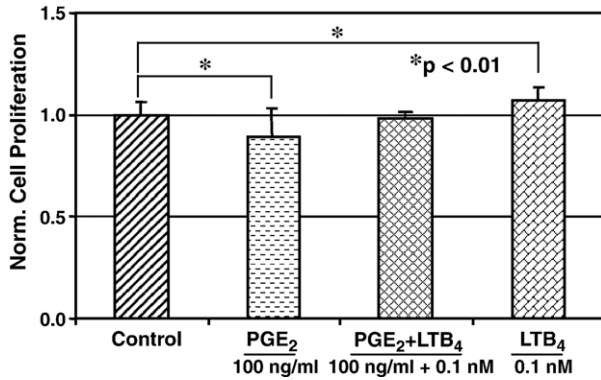


Fig. 2. The combined effect of PGE₂ (100 ng/ml) and a low dose of LTB₄ (100 pM) on human patellar tendon fibroblasts. The addition of PGE₂ significantly decreased and the addition of LTB₄ at 100 pM significantly increased the cell proliferation. However, the combined addition of both agents brought the proliferation level back to that of untreated cells. Four independent experiments were carried out; total sample size was 12 for each group.

LTB₄ and PGE₂ used in this study did not cause apparent changes in the morphology of tendon fibroblasts in culture, which suggests that the possible toxic effects of these two agents on human tendon fibroblasts were minimal (data not shown).

3.2. LTB₄ negated the MMP-1 and MMP-3 gene expression induced by PGE₂

PGE₂-mediated catabolic effect on matrix proteins has been well documented (Varga et al., 1987). We observed the counteraction of low concentration of LTB₄ against PGE₂ in cell proliferation. Next we examined the potential antagonistic effect of LTB₄ on the catabolic action mediated by PGE₂ by examining the expression and production of MMP-1 and MMP-3 in the presence of LTB₄ alone and in combination with PGE₂. Compared to the control level, treatment of HPTFs for 48 h with 100 ng/ml PGE₂ increased MMP-1 gene expression whereas LTB₄ at 0.1 nM decreased it. Interestingly, the addition of a

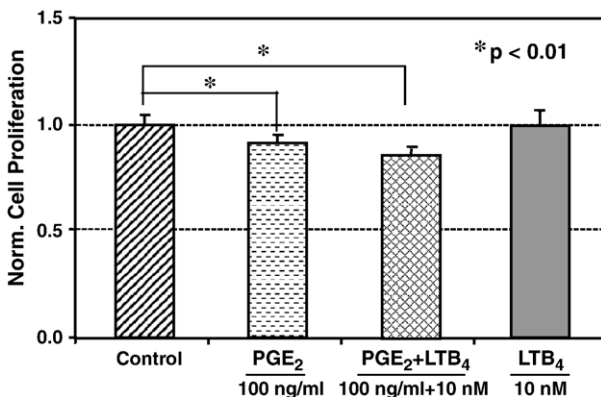


Fig. 3. The combined effect of PGE₂ (100 ng/ml) and a high dose of LTB₄ (10 nM) on human patellar tendon fibroblasts. The addition of PGE₂ decreased cell proliferation and the addition of LTB₄ at 10 nM did not change the cell proliferation compared to that of untreated cells. When both agents were added in combination, cell proliferation further decreased compared to the already reduced level of cell proliferation brought by PGE₂ alone. Two independent experiments were done, with a total sample size of 6 for each group.

low concentration of LTB₄ (0.1 nM) significantly down-regulated 50% the expression of MMP-1 (Fig. 4A and B). Furthermore, the addition of LTB₄ appeared to counteract the stimulatory action of PGE₂ on MMP-1 expression, down-regulating close to the control level.

We further analyzed for MMP-1 protein production after stimulation with PGE₂ alone or in combination with LTB₄. As seen in mRNA level, MMP-1 protein production was correspondingly increased by 103% by 100 ng/ml PGE₂ compared to the control whereas LTB₄ at 0.1 nM slightly decreased the level by 20% compared to the control. A combined treatment with 0.1 nM LTB₄ and 100 ng/ml PGE₂ brought the MMP-1 level back to control (Fig. 4C). Collectively, our results suggest that the gene expression and the corresponding protein secretion of MMP-1 are regulated by PGE₂ and LTB₄ and low concentration of LTB₄ can rescue PGE₂-mediated stimulation of MMP-1 in HPTFs.

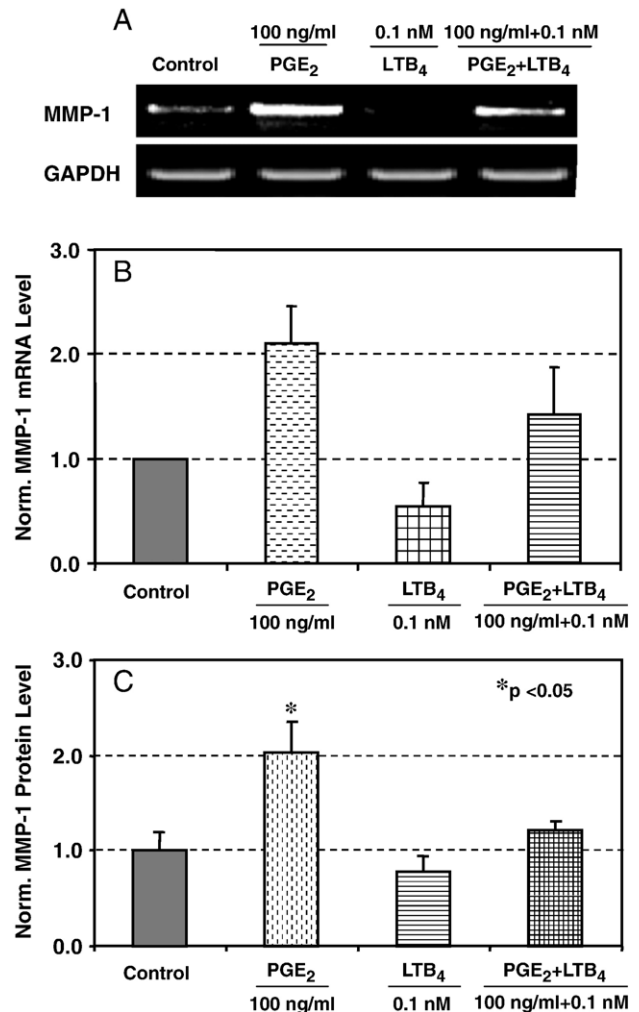


Fig. 4. Effects of PGE₂ and LTB₄ on MMP-1 mRNA expression and protein production. A. Representative RT-PCR result from three independent experiments using cells from two different donors. B. The combined densitometric data from three independent experiments. The data show that PGE₂ induced MMP-1 and LTB₄ decreased it compared to the control; a combined treatment of PGE₂ and LTB₄ brought the MMP-1 mRNA level back close to the control. C. PGE₂ treatment increased MMP-1 protein level whereas LTB₄ reduced it compared to the control. Two independent experiments were performed with a total sample size of 6.

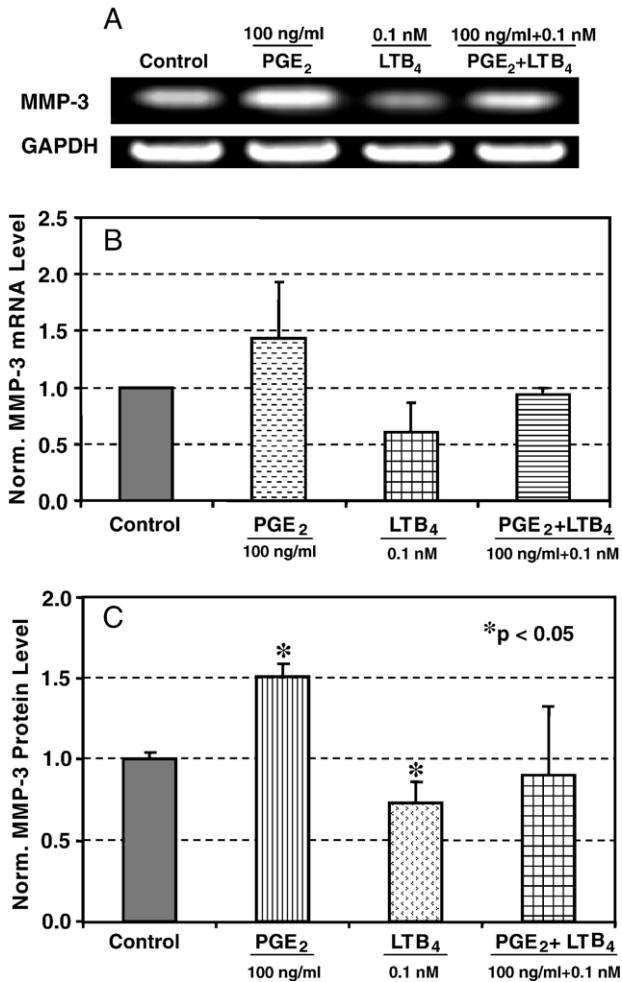


Fig. 5. Effects of PGE₂ and LTB₄ on MMP-3 mRNA expression and protein production. A. A representative RT-PCR result from three independent experiments using cells from two different donors. B. The combined densitometric data from three independent experiments. The data show that MMP-3 mRNA level was markedly increased by PGE₂ compared to the control while it was reduced by LTB₄ compared to the control. A pretreatment with LTB₄ for 30 min prior to PGE₂ treatment brought the levels back to the control level. C. MMP-3 protein production also increased by PGE₂ treatment and decreased by LTB₄, while combined treatment brought the levels close to the control. Two independent experiments were performed with a total sample size of 6.

We also examined the effect of LTB₄ and PGE₂ on the gene expression and protein secretion of MMP-3. A similar pattern to MMP-1 in response to PGE₂ and LTB₄ treatments was observed (Fig. 5A, B, and C). Specifically, MMP-3 gene expression was increased by 43% with PGE₂ treatment compared to the

untreated, while LTB₄ at 0.1 nM decreased the level by 40% compared to the control level. Again, pretreatment with LTB₄ prior to PGE₂ treatment brought the MMP-3 mRNA level back to the control levels (Fig. 5B). The level of MMP-3 protein production also was augmented by treatment with 100 ng/ml PGE₂. Specifically, the MMP-3 level was increased by 50% in response to PGE₂ while LTB₄ at 0.1 nM decreased it by 30%. However, the combined treatment brought the MMP-3 protein production back close to the control level (Fig. 5C).

3.3. PGE₂ and its combination with LTB₄ did not affect collagen type I gene expression

The effect of increasing concentrations of PGE₂ and the potential anabolic action of LTB₄ at low concentrations on collagen I gene expression was determined. Treatment with 100 ng/ml PGE₂, or higher doses (250 and 500 ng/ml), did not change collagen type I mRNA gene expression (Fig. 6A). LTB₄ at 0.1 nM combined with 100 ng/ml PGE₂ did not affect collagen type I mRNA gene expression either (Fig. 6B).

4. Discussion

This study showed that exogenous LTB₄ affects HPTF proliferation in a dose-dependent manner. Specifically, low concentrations of LTB₄ (≤1 nM) exerted a positive effect on the proliferation of HPTFS. Although there is no published study on the effects of LTB₄ on human tendon fibroblasts, our results agree with previous reports obtained using other cell/tissue types. For example, LTB₄ was shown to stimulate DNA synthesis in cultured human epidermal keratinocytes at picomolar concentrations (Kragballe et al., 1985). Also, in arterial smooth muscle cells, LTB₄ at 10 pM was shown to stimulate cell proliferation and DNA synthesis (Palmberg et al., 1987, 1989).

Leukotrienes exert their effect primarily via their receptors, which elicit a cascade of signaling events (Denzlinger, 1996). The mechanism of LTB₄-mediated proliferative response is thought to involve protein kinase C (PKC) activation and the stimulation of early response genes such as topoisomerase I and nuclear factor kappa-B (NFκ-B) (Mattern et al., 1990; Brach et al., 1992). In addition, there are interactions between growth stimulatory cytokines such as IL-6, which could potentially mediate LTB₄-mediated proliferative effects (Brach et al., 1992; Rola-Pleszczynski and Stankova, 1992; Denzlinger, 1996). The optimal concentration for the leukotriene-induced cytokine production is shown to be in the sub-micromolar range (Rola-Pleszczynski and Stankova, 1992).

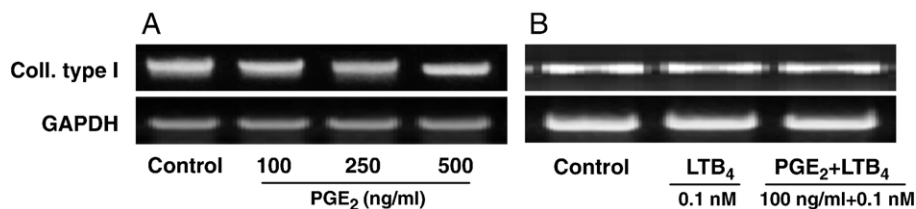


Fig. 6. Effect of PGE₂ and LTB₄ on collagen type I gene expression in HPTFs. A. PGE₂ did not affect collagen type I gene expression. B. The combined treatment with LTB₄ did not affect collagen type I gene expression either. Three independent experiments were performed using cells from two different donors.

This study also showed that PGE₂ at 100 ng/ml significantly decreased HPTF proliferation, which is consistent with the result of our previous report (Cilli et al., 2004). Furthermore, low concentrations (≤ 1 nM) of LTB₄ negated the inhibitory effect of PGE₂ on HPTF proliferation. However, LTB₄ at higher concentrations (>1 nM) was not able to counteract the PGE₂ effect; on the contrary, it appeared to further decrease the cell proliferation when combined with PGE₂. These results may have important implications in tendon homeostasis as well as in the development of tendinopathy. The potential scenario would be that at low doses, LTB₄ is beneficial in terms of negating the catabolic effect of PGE₂ on fibroblast proliferation and thereby maintaining the tendon homeostasis in vivo. However, at high concentrations, LTB₄ may worsen the catabolic effect of PGE₂ on fibroblast proliferation. Consequently, there would not be enough tendon fibroblasts to maintain tendon matrix homeostasis. The small reduction in cell proliferation by PGE₂ may also imply that the development of tendinopathy takes time, which is known to be an insidious process over a long period of time (Almekinders, 1998).

The current study showed that PGE₂ significantly increased the gene expression and protein production of MMP-1 and MMP-3. Our results are consistent with the previous observation that exogenous PGE₂ up-regulates MMPs in gingival fibroblasts (Sakaki et al., 2004). Other studies have reported that PGE₂ mediates IL-1 β -induced MMP-1 up-regulation in tendon and gingival fibroblasts (Domeij et al., 2002; Tsuzaki et al., 2003). It is noted that tendon consists predominantly of collagen type I with a triple helical structure that resists general proteolytic degradation but is susceptible to MMPs. MMPs are produced as inactive zymogens and their extracellular activation can be initiated by various factors such as plasminogens or other active MMPs (Visse and Nagase, 2003). Although MMP-3 does not degrade collagen type I, it degrades collagen-associated small proteoglycans, elastin, fibronectin, gelatin, and other types of collagens (Birkedal-Hansen, 1993). In addition, MMP-3 has been shown to activate MMP-1 (Murphy et al., 1987) and other collagen type I-degrading MMPs, including MMP-8 and MMP-13 as well as MMP-7, which is also involved in the activation of MMP-1 (Visse and Nagase, 2003). Therefore, the upregulated MMP-3 by PGE₂ may enable MMP-1 and/or other collagen-degrading enzymes to efficiently access collagen molecules for digestion in vivo.

It is interesting to note that neither PGE₂ nor LTB₄ nor their combination at the doses used in this study changed collagen gene expression (Fig. 6). These results suggest that PGE₂-mediated inhibition of collagen production, as shown in our previous study (Cilli et al., 2004), is perhaps via the induction of collagen-degrading enzymes such as MMP-1 and MMP-3. Another possibility for the decrease in collagen production by PGE₂ is that PGE₂ affects collagen mRNA stability. Although it has been shown in human dermal fibroblasts that PGE₂ did not alter the stability of procollagen I and III mRNA (Varga et al., 1987), the potential modulation of mRNA stability should be examined further, especially since PGE₂ effects are highly cell and tissue specific (Serhan and Levy, 2003).

We are aware of a few limitations of this study. First, we did not determine the kinetic effect of LTB₄ on HPTF proliferation and collagen synthesis. Also, we do not know if the decreased cell numbers mediated by either PGE₂ alone or in combination with high concentrations of LTB₄ are due to apoptosis or cell growth arrest. We did not look into the potential mechanistic cellular events by which LTB₄ and PGE₂ regulate collagen synthesis and the expression/production of MMPs. The molecular mechanisms by which the low concentration of LTB₄ exerts anti-catabolic action against PGE₂ and the potential molecular interplay between PGE₂ and LTB₄ will be of special interest and future studies are required. In addition, it is known that LTB₄ and PGE₂ actions are mediated via their receptors, e.g., BLT1 and BLT2 (Tager and Luster, 2003) and EP1, EP2, EP3, and EP4 (Negishi et al., 1995; Narumiya et al., 1999), respectively. Thus, it is important to investigate the expression and/or activation of their cognate receptors and the signaling pathways for better understanding the precise role of dose-dependent counter-activity of LTB₄ on PGE₂ in tendon homeostasis in future studies.

In conclusion, this study showed that LTB₄ at sub-nanomolar concentrations increases tendon fibroblast proliferation and negates the catabolic effects of PGE₂. The results suggest that although LTB₄ is mainly pathogenic in nature, low levels are actually beneficial in maintaining tendon homeostasis by regulating tendon fibroblast proliferation and MMPs, which in turn control matrix turnover. The ability of LTB₄ to counteract the catabolic action of PGE₂ may thus play an important role in delaying the development of tendinopathy. A better understanding of the precise mechanisms by which LTB₄ regulates the effects of PGE₂ on tendon fibroblast proliferation and MMP expression will aid in developing effective therapeutic approaches for prevention as well as treatment of tendinopathy.

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