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Prostaglandin E2 and Prostaglandin F2α Differentially Modulate Matrix Metabolism of Human Nucleus Pulposus Cells

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Abstract

Prostaglandin (PG) actions on disc metabolism are unclear even though certain PGs are highly expressed by disc cells under inflammatory conditions and nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used to block PG production to treat back pain. Hence this study aimed to (1) quantify gene expression of arachidonic acid cascade components responsible for PG synthesis and (2) examine the effects of key PGs on disc matrix homeostasis. Microarray analysis revealed that inflammatory stress increases expression of synthases and receptors for prostaglandin E2 (PGE_2) and prostaglandin F2α ($PGF_{2\alpha}$), resulting in elevated PGE_2 and $PGF_{2\alpha}$ production in conditioned media of disc cells. PGE_2 diminished disc cell proteoglycan synthesis, in a dose-dependent manner. Semiquantitative RT-PCR revealed differential effects of PGE_2 and $PGF_{2\alpha}$ on disc cell expression of key matrix structural genes, aggrecan, versican, collagens type I and II. PGE_2 and $PGF_{2\alpha}$ also decreased message for the anabolic factor, IGF-1. PGE_2 decreased mRNA expression for the anti-catabolic factor TIMP-1 while $PGF_{2\alpha}$ increased mRNAs for catabolic factors MMP-1 and MMP-3. Thus, PGE_2 and $PGF_{2\alpha}$ may have an overall negative impact on disc matrix homeostasis, and the use of NSAIDs may impact disc metabolism as well as treat back pain.

Keywords

nucleus pulposus cells; prostaglandins; PGE_2; $PGF_{2\alpha}$; intervertebral disc matrix

Low back pain is often associated with intervertebral disc degeneration (IDD) and remains a significant health problem.[1] Etiology of IDD is complex and still not well understood.[2]

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Thus, current treatment options often address symptoms without an understanding of the mechanism of action. For example, nonsteroidal anti-inflammatory drugs (NSAIDs) targeting prostaglandin (PG) production are frequently prescribed for their analgesic and anti-inflammatory effects. However, few studies have addressed potential effects of PG on disc cell metabolism.

Several lines of evidence demonstrate the presence of PGs in discs under stress and implicate the possible involvement of PGs in IDD progression. The pro-inflammatory cytokines IL-1 and TNF-α are increased in degenerated human discs[3,4] and have multiple actions that may contribute to disc matrix loss.[5,6] Both cytokines induce cyclooxygenase 2 (COX-2), a key enzyme for PG biosynthesis, in disc cells with subsequent synthesis of PGs.[7–10] COX-2 expression and PG synthesis in disc cells are also induced by mechanical stress, another predisposing factor that can disrupt disc structure and initiate the degenerative cascade.[11,12] PGE_2, a major inflammatory PG mediator associated with sensitization of proprioceptive neurons and involved with pain induction, is elevated in herniated lumbar discs.[13]

The presence of PGs in disc cells suggests they have functional roles in disc metabolism, but current evidence is indirect, limited, and at times contradictory. Chemical inhibition of COX-2 partially dampens the IL-1-mediated induction of catabolic response in human disc cells.[14] In this study, COX-2 inhibition also diminishes IL-1-mediated suppression of proteoglycan sysnthesis. In contrast, Yoo et al.[15] found that proteoglycan synthesis by canine nucleus pulposus (NP) cells was inhibited by NSAIDs that inhibit both the constitutive COX-1 and inducible COX-2. This suggested that either endogenously produced PGs actually supported matrix proteoglycan synthesis, or that the NSAIDs decrease proteoglycan synthesis through nonspecific actions unrelated to PGs under these experimental conditions. On the other hand, Karppinen et al.[16] treated pigs with stab-induced disc degeneration with tiaprofenic acid or indomethacin and reported no negative effects on matrix metabolism from long-term administration of these NSAIDs in this in vivo model. Iwabuchi et al.[17] measured the effect of PGE_2 on rat disc wet weights and found no significant difference in weight loss, except in those discs concurrently treated with low-intensity pulsed ultrasound where PGE_2 diminished disc weight loss, for example, PGE_2 was matrix protective. Though indirect, these findings implicate regulatory roles of PGs in disc matrix homeostasis.

The minimal exploration of PG actions on intervertebral disc cells contrasts with the many studies exploring the role of PGs, in particular PGE_2, in chondrocyte metabolism, as it relates to the pathophysiology of arthritis (reviewed in Refs.[18,19]). Depending on the dose, time, and experimental model, PGE_2 actions are diverse and include induction of chondrocyte apoptosis, reversal of proteoglycan degradation induced by IL-1, stimulation of aggrecan synthesis, increasing or decreasing collagen synthesis, inhibition of DNA synthesis or increasing growth, and sensitizing chondrocytes to the anabolic actions of IGF-1. Goldring et al. have defined the mechanisms through which PGE_2 inhibits type I collagen gene expression and stimulates type II collagen gene expression in chondrocytes and fibroblasts [20,21].

Given the similarities between chondrocytes and cells of the NP, and the frequency with which patients with low back pain are treated with NSAIDs, it is appropriate to determine the potential of human NP (hNP) cells for PG synthesis and response to PGs. The current studies approach this problem through (1) microarray analysis for components of the arachidonic acid cascade, and (2) determining the response of hNP to exogenous PGE_2 and $PGF_{2\alpha}$.

MATERIALS AND METHODS

Nucleus Pulposus Cell Isolation and Culture

The NP was dissected from patient disc surgical specimens (19–59 years, mean = 42.2 years, and average Thompson degeneration grade 2–3.[22] All of these NP tissues were isolated from patients underwent surgeries for disc degeneration and not disc herniation, and therefore contact between these tissues and cells outside of the disc, that is, macrophages, was minimal or nonexistent. The experimental protocol was approved by the human subjects Institutional Review Board at the University of Pittsburgh. Cells were isolated and cultured in F-12/D-MEM containing 10% FCS, 1% PS, and 25 μg/ml L-ascorbic acid under standard conditions (37°C, 5% CO_2, 95% air, bicarbonate buffering to maintain pH 7.2) as previously described.[23] Cell culture materials were purchased from Invitrogen/Gibco (Carlsbad, CA) unless noted otherwise, and the hNP cells were used at passage 1 or 2.

Microarray Analysis of NP Gene Expression

hNP cells (grade 3) at passage 1 were exposed to 0 and 5 ng/ml TNF-α for 24 h. Total RNA was isolated and hybridized to Affymetrix GeneChip Human Genome U133 Plus 2.0 Arrays designed for analysis of 47,000 transcripts, following standard procedures recommended by the manufacturer. Array data were collected from three independent experiments and analyzed using RMA[24] and Limma[25] in the R statistical environment. Expression levels are averages from $n = 3 \pm 1$ SE.

Exposure to Prostaglandins

hNP cells were isolated and expanded in monolayer, and passed to three-dimensional culture in alginate beads as previously described.[23] Experiments were begun after the cells had been in three-dimensional culture for at least 1 week and the response of hNP cellsto PGE_2 or $PGF_{2\alpha}$ (Sigma–Aldrich, St. Louis, MO) in low serum (1%) media was evaluated after 3 days exposure. The concentrations used (100 ng/ml PGE_2 and 75 ng/ml $PGF_{2\alpha}$) were chosen based on the medium concentrations attained in response to 1 ng/ml IL-1 (104 ± 14 and 62 ± 9 ng/ml for PGE_2 and $PGF_{2\alpha}$, respectively). Cells were also exposed to 10 μM butaprost (a specific PG PTGER2 receptor agonist) and 50 ng/ml fluprostenol (a metabolically stable analog of $PGF_{2\alpha}$ with potent PTGFR receptor agonist activity, Sigma–Aldrich) for 3 days. Total RNA were extracted using the RNeasy Mini Kit (Qiagen, Germantown, MD), and DNA measured by Picogreen (Molecular Probes, Eugene, OR) after digestion in papain buffer.[23]

Matrix Protein Synthesis

Proteoglycan synthesis was measured on day 3 and total protein/collagenase sensitive incorporation of ^{3}H proline into proteins was done from 48 to 72 h of PG activation. Proteoglycan synthesis was measured from a 6 h pulse labeling with ^{35}S-sulfate at 20 μCi/ml as previously described[23] and the results calculated as pmol ^{35}S incorporated/μg DNA and expressed relative to control. Collagenase sensitive and total protein synthesis was measured as 24 h ^{3}H-Proline incorporation as described,[26] calculated as dpm/μg DNA, and expressed relative to untreated control.

Gene Expression

Gene expression was analyzed by real-time RT-PCR using Bio-Rad iCycler IQ4 detection system. The reactions were done with the validated primers (Table 1). Real-time RT-PCR reactions were done in duplicate in 96-well plates in a volume of 25 μl using the reagents and protocol per the Bio-Rad iScript One-Step RT-PCR Kit (Hercules, CA). The cycle threshold (C_t) values were obtained, and data normalized to GAPDH expression using the $\Delta\Delta C_t$ method to calculate relative mRNA levels compared to untreated samples.

Statistical Analysis

Data are given as mean ± standard error, and *p*-value of Student's *t*-test were calculated, and a *p*-value of <0.05 defined statistically significant differences.

RESULTS

Microarray Analysis

Microarray analysis was done to assess the expression of the components of the arachidonic acid cascade (Table 2). Typically considered a constitutively expressed gene, COX-1 mRNA (67 ± 25) is low compared to inducible COX-2 (206 ± 37) in hNP cells under basal and TNFα-induced conditions. mRNA levels of prostaglandin E synthase genes, all three isoforms, are also high, especially PTGES3 which is induced by TNFα ~3-fold. Synthases for $PGF_{2\alpha}$ and prostacyclin synthase (PTGIS) are also highly expressed. Receptors for PGE_2, in particular PTGER2 and 4 are expressed at high basal levels and are upregulated ~2-fold by TNFα. Receptor for $PGF_{2\alpha}$ is also expressed at a moderate level and upregulated by TNFα. Synthetases responsible for synthesis of PGE_2, $PGF_{2\alpha}$, and their receptors were upregulated by TNF-α, suggesting that PGE_2 and $PGF_{2\alpha}$ are important PGs in disc metabolism. These results are consistent with previous findings of high levels of PGE_2 in herniated lumbar discs[13] and high $PGF_{2\alpha}$ production by hNP cells[14] as noted in the Materials and Methods Section. The high expression of synthases and receptors for PGE_2 and $PGF_{2\alpha}$, and their documented secretion by hNP in response to both IL-1 and TNF-α directed our choice to evaluate hNP cell response to these two PGs in this study.

Response of NP Cells to PGE_2 and $PGF_{2\alpha}$

PGE_2 but Not $PGF_{2\alpha}$ Decreases Proteoglycan Synthesis—PGE_2 at 100 ng/ml significantly decreased total proteoglycan synthesis by ~30% after 24 or 72 h exposure; in contrast, $PGF_{2\alpha}$ did not affect total proteoglycan synthesis (Fig. 1A). No effects were seen at

lower concentrations of $PGF_{2\alpha}$ (data not shown). mRNA for two major aggregated proteoglycans, aggrecan core protein and versican, were also determined. As seen in Figure 1B, PGE_2 decreased aggrecan mRNA to 46% while increasing versican mRNA to 173% of control. In contrast, $PGF_{2\alpha}$ increased aggrecan message to 148% while decreasing versican mRNA to 75% of control.

PGE_2 but Not $PGF_{2\alpha}$ Increase hNP Collagen Synthesis—As seen in Figure 2, neither PGE_2 nor $PGF_{2\alpha}$ significantly changed ^3H proline incorporation into total proteins. However, there were modest increases in collagen synthesis in the presence of PGE_2 or 10 μM butaprost, a specific PTGER2 receptor agonist. Neither $PGF_{2\alpha}$ nor 50 ng/ml fluprostenol, a metabolically stable analog of $PGF_{2\alpha}$ with potent PTGFR receptor agonist activity, significantly affected collagen or total protein synthesis. Figure 3 shows that, in contrast with its effects on total collagen synthesis, PGE_2 actually decreased message for both collagens type I and II by ~ 65% and 40%, respectively. However, it did modestly increase mRNA for collagen XV by 25%. 100 ng/ml PGE_2 also exerted similar control on gene expression of these collagens after 24 h exposure (0.35 ± 0.013 for collagen I, 0.55 ± 0.05 for collagen II, 0.91 ± 0.32 for collagen VIII, and 1.85 ± 0.41 for collagen XV). The only significant effect of $PGF_{2\alpha}$ on message for these four types of collagen was to increase mRNA for Collagen XV by twofold. Thus, PGE_2 and $PGF_{2\alpha}$ exert distinct actions on gene expression and synthesis of disc matrix collagen and proteoglycans.

PGE_2 and $PGF_{2\alpha}$ Differentially Regulate Expression of Growth Factors and Catabolic Genes—The effects of these PGs on the expression of growth factors known to modulate matrix protein synthesis and some of the TIMPs and MMPs involved in matrix protein degradation are shown in Figure 4. Neither PGE_2 nor $PGF_{2\alpha}$ affected mRNA for TGF-β but had similar effects on IGF-1 mRNA (decrease by ~30%). MMP-3 and MMP-1 mRNA were increased two- to threefold by $PGF_{2\alpha}$ while PGE_2 decreased mRNA for MMP-1 to ~15% of control values. PGE_2 decreased (to 50% of control) while $PGF_{2\alpha}$ increased (to 161% of control) mRNA of the anti-catabolic gene, TIMP-1.

DISCUSSION

This study evaluated the induction of constituents of the arachidonic acid pathway in response to inflammatory cytokines in order to identify key control points. The microsomal prostaglandin E synthase 1 (PTGES) is induced (~3-fold) in hNP by TNF-α, as it is in articular chondrocytes by this and other inflammatory cytokines[27] as well as by mechanical stress.[28] PTGES has been implicated in the development of arthritis and suggested as a therapeutic target in this joint disease. However, given the much higher, albeit stable amount of message of the constitutively expressed cytosolic PTGES3 in these disc cells, further studies are needed to determine the actual synthase protein and activity responsible for PGE_2 synthesis in the disc and to conclude which synthase could potentially be targeted to diminish PG production in IDD.

PTGFS gene expression is actually greater than PTGES under basal conditions and is also increased (~2-fold) to a similar level as that of PTGES in TNF-α-activated hNP cells (Table 2). Thus, cytokine activation of PG production works through increasing both COX-2 and

the downstream PG synthases. Aside from a study performed by Jacob et al.[29] to test the ability of $PGF_{2\alpha}$ to alter chondrocyte matrix protein production, this is the first report, to the best of our knowledge, of the expression of genes responsible for the production of $PGF_{2\alpha}$ as well as response of NP cells to this PG.

PTGIS converts PGH_2 into prostacyclin (PGI_2), a potent vasodilator and inhibitor of platelet aggregation with roles in acute and chronic inflammation. mRNA of PTGIS is present in hNP, but in contrast with the synthases for PGE_2 and $PGF_{2\alpha}$, it is actually decreased to 1/3 of basal levels in TNF-α-activated cells. The relevance of this change in hNP cell function remains to be tested, as very low levels of receptor mRNA for prostacyclin are identified. mRNA for receptors of both PGE_2 and $PGF_{2\alpha}$ are present and increase two- to threefold in cytokine-activated cells, thus, suggesting that PGE_2 and $PGF_{2\alpha}$ play prominent roles in discs.

This study investigated the action of specific PGs, that is, PGE_2 and $PGF_{2\alpha}$, on disc cells that show potential to alter the matrix composition in ways that could affect both structure and function. PGE_2 decreased aggrecan mRNA to 46% of control while decreasing proteoglycan synthesis to ~70% of control. $PGF_{2\alpha}$, while not affecting total proteoglycan synthesis, also changed the relative gene expression of aggrecan and versican, but in the opposite direction as compared to PGE_2, that is, increased aggrecan while decreasing versican. Our results with disc cells differ from those of Jakob et al.[29] who evaluated the actions of specific PGs after longer exposure (2 weeks) on adult articular chondrocyte pellets and found no effect of PGE_2 but a twofold increase in GAG/DNA content in $PGF_{2\alpha}$-treated cells. This is consistent with evidence of other phenotypic differences between disc cells and articular chondrocytes. These data suggest that the predominant type of disc proteoglycans may very well be affected by PG exposure, but the consequences for disc matrix structure and function remain to be explored.

Neither PG affected total protein synthesis, however, PGE_2 and the PTGER2 agonist butaprost increased collagen synthesis, suggesting this action of PGE_2 may be through the PTGER2 receptor. PGE_2 decreased message for both collagen I and II and only modestly increased that for collagen XV. Because collagen synthesis represents all collagen gene products, it is not surprising that increased or decreased collagen synthesis in response to PGE did not reflect the same changes in expression of a few collagen types such as type I and II. Indeed, regulation of collagen synthesis is enormously complex which is often involved the coordination of many collagens, each of which is under multiple steps of gene expression control in a temporal manner.[30,31] Our findings suggest that either additional collagen types are being induced that were not identified in our RT-PCR analysis or additional posttranscriptional regulations of collagen I and II by PGE_2 exist within the disc cells. The decrease in collagen I is consistent with that documented in chondrocytes[19,20] treated with PGE_2; the decrease in message for collagen II is not, as PGE_2 is reported to have no effect[29] or to actually increase[20] collagen II mRNA. However, the actions of PGE_2 on chondrocyte collagen metabolism can be dose dependent with low concentrations increasing and high concentrations decreasing synthesis.[32]

The lack of any significant effect of $PGF_{2\alpha}$ or its nonmetabolizable analog fluprostenol on total protein and collagen synthesis was surprising, as it is frequently associated with anabolic processes in muscle cells.[33,34] Furthermore, it is reported that while $PGF_{2\alpha}$ had no effect on collagen I, it did enhance collagen II expression by ~20× in articular chondrocytes.[29] Of the parameters evaluated in the current study of hNP, only message for collagen XV was significantly affected by $PGF_{2\alpha}$. Collagen XV, a nonfibrillar collagen, exhibits diverse conformations and supramolecular assemblies and has many sites of attachment for glycosaminoglycan chains. In addition to its structural role, this collagen has been implicated in migration, proliferation, apoptosis, or survival of different cell types.[35,36] If the changes we observed in expression of collagens are translated into changes in the collagen composition of disc matrix proteins, PGs show potential to alter the matrix composition of the disc in ways that could affect both structure and function. Therefore, future studies should address the substrates of collagenases synthesized in response to PG exposure.

In addition to their influence on matrix gene expression and synthesis, these PGs also modulated the expression of factors that can affect disc cell matrix metabolism. Both PGE_2 and $PGF_{2\alpha}$ decreased message for IGF-1 by 29% and 32%, respectively. As IGF-1 mediates chondrocyte cell survival,[37] rescues annulus cells of the disc from senescence,[38] and increases disc matrix synthesis,[39] one could postulate that chronic exposure to PGs might have a role in the loss of disc cell viability and function through their downregulation of this growth factor. Prostaglandin E1 was recently reported to induce the production of epidermal growth factor, a factor important in cell proliferation and cell survival, in cultured human annulus cells.[40] Thus, PGs appear to have different influences on disc cell metabolism, including matrix metabolism as well as cell growth and survival.

$PGF_{2\alpha}$ and PGE_2 also modulate disc matrix catabolic balance through their influence on MMP and TIMP gene expression. Although PGE_2 decreases TIMP-1, it decreases message for MMP-1 to an even greater extent (51% vs. 85% decrease from Control) while not affecting message for MMP-3. $PGF_{2\alpha}$, in contrast, increases both MMP-1 and MMP-3 (~3-fold) while increasing TIMP-1 only 60%. We found no effect of either PG on expression of TIMP-3, however, the effects, if any, on other MMPs and/or aggrecanase expression as well as activity would need to be evaluated before concluding which PG would facilitate the greatest net matrix degradation.

In conclusion, defining effects of specific PGs on disc cell metabolic activities could facilitate targeting specific synthases and/or receptors to protect the disc from degeneration. Based on the chosen endpoint measurements, we conclude that during clinical use of NSAIDs, PGE_2 might have greater net effect than $PGF_{2\alpha}$ on anabolic processes while $PGF_{2\alpha}$ primarily increases catabolic activities. As both modulate expression of matrix proteins in a manner that could alter the ratios of the various proteoglycans and collagens in the matrix, the consequences for disc structure and function remain to be defined. Studies evaluating the ability of COX-2 inhibitors and/or NSAIDs to modulate hNP response to inflammatory cytokines would help to predict the changes in matrix homeostasis when these agents are used.

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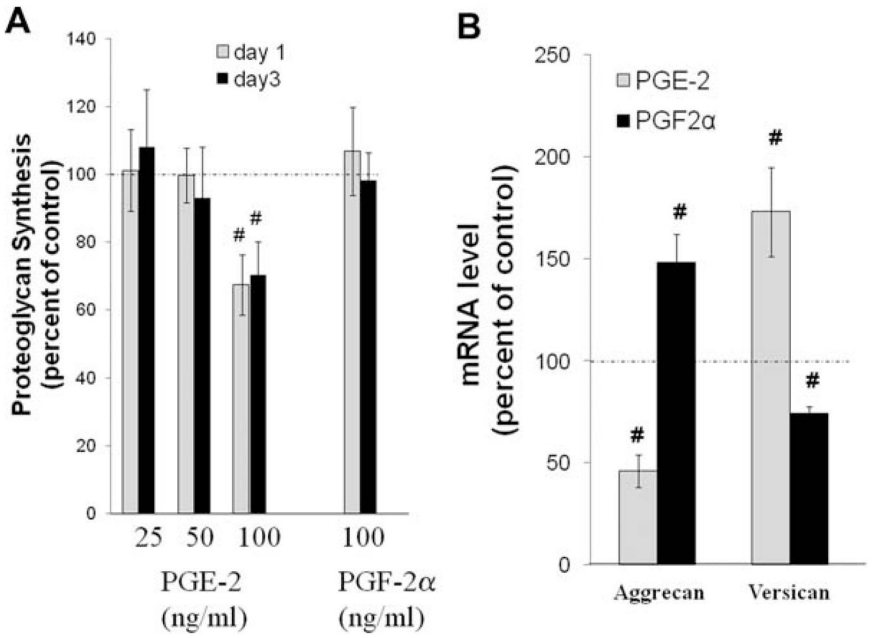

Figure 1.

Effects of PGE_2 and $PGF_{2\alpha}$ on proteoglycan gene expression and synthesis. (A) Effects of PGE_2 and $PGF_{2\alpha}$ on total proteoglycan synthesis. hNP in alginate beads were treated with a range of PGE_2 concentrations or 75 ng/ml $PGF_{2\alpha}$ as described in the Materials and Methods Section and pulse labeled with ^{35}S-Sulfate to determine relative rates of total proteoglycan synthesis. Values are mean ± SE of $n = 4–8$. $^{\#}p < 0.05$ versus Control. (B) Effects of PGE_2 and $PGF_{2\alpha}$ on mRNA expression of two major proteoglycans, aggrecan and versican. HNP in alginate beads were exposed to 100 ng/ml PGE_2 or 75 ng/ml $PGF_{2\alpha}$ for 72 h, RNA isolated, and rtPCR done to evaluate mRNA for aggrecan and versican as described in the Materials and Methods Section. Values are mean ± SE of $n = 8–10$. $^{\#}p < 0.05$ versus Control. Results demonstrated that PGE_2 and $PGF_{2\alpha}$ differentially increase and decrease mRNA for aggrecan and versican.

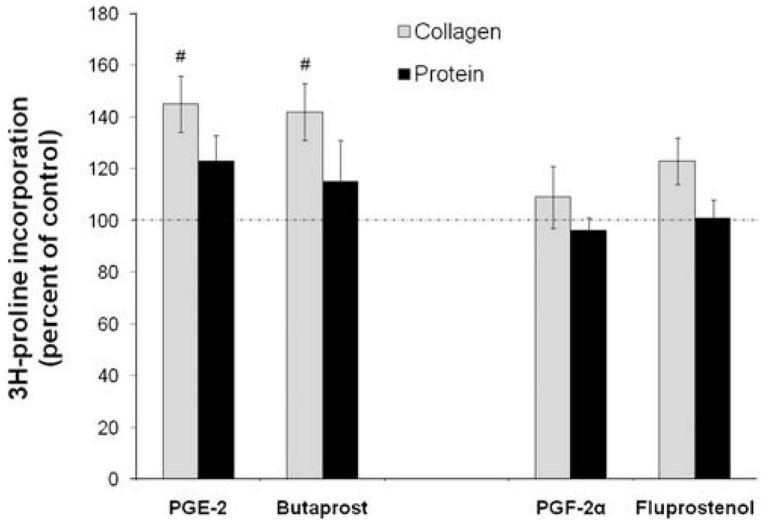

Figure 2.

PGE_2 and the PTGER2 receptor agonist butaprost increase hNP collagen, but not total protein synthesis. HNP were exposed to 100 ng/ml PGE_2, 10 μM butaprost, 75 ng/ml $PGF_{2\alpha}$, or 50 ng/ml fluprostenol for 72 h with ^{3}H proline added for the final 24 h of culture. Values are mean ± SE of $n = 4–6$. $^{\#}p < 0.05$ versus Control.

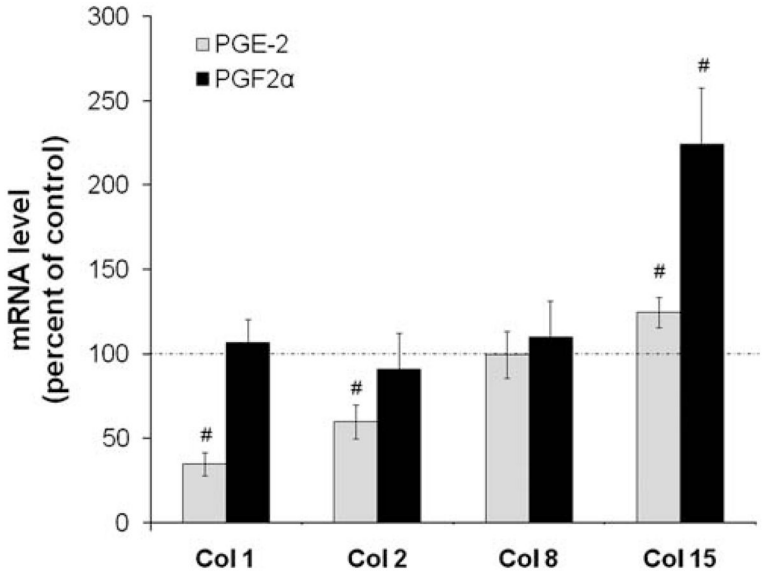

Figure 3.

PGE_2 and $PGF_{2\alpha}$ differentially increase and decrease mRNA for collagen I, II, VIII, and XV. HNP in alginate beads were exposed to 100 ng/ml PGE_2 or 75 ng/ml $PGF_{2\alpha}$ for 72 h, RNA isolated, and rtPCR done to evaluate expression of collagens as described in the Materials and Methods Section. Values are mean ± SE of $n = 6–10$. $^{\#}p < 0.05$ versus Control.

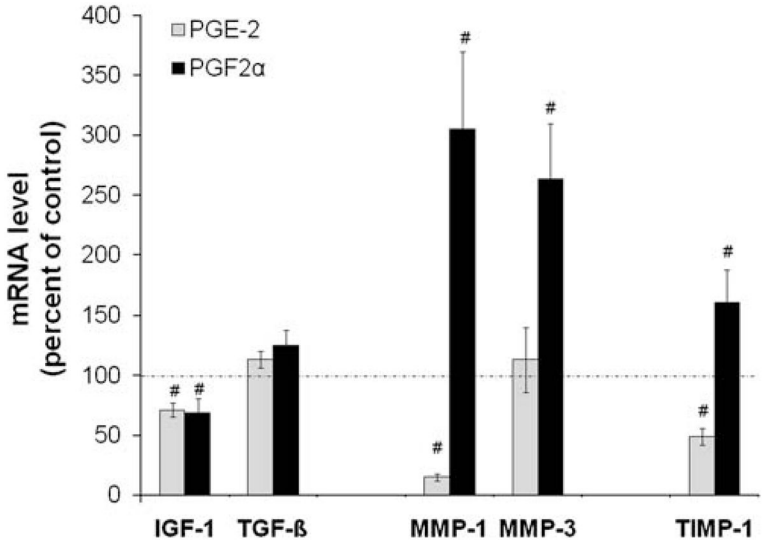

Figure 4.

PGE_2 and $PGF_{2\alpha}$ variably increase and decrease mRNA for factors that modulate disc matrix synthesis and degradation. hNP in alginate beads were treated as described for Figure 2, RNA isolated, and rtPCR done as described in the Materials and Methods Section. Values are mean ± SE of $n = 6–10$. $^{\#}p < 0.05$ versus Control

Table 1

Primers Used in Real-Time RT-PCR Analysis of Gene Expression

Gene	Forward (5′–3′)	Reverse (5′–3′)
Aggrecnn	AAGAATCAAGTGGAGCCGTGTGTC	TGAGACCTTGTCCTGATAGGCACT
Verslcan	GCGGAGACCAGTGTGAACTTGATT	ACATAACTTGGAAGGCAGAGGCAC
Col la 2	GGAAACAGACAAGCAACCCAAACT	GGTCATGTTCGGTTGGTCAAAGATAA
Col 2a 1	ATGACAATCTGGCTCCCAAC	GAACCTGCTATTGCCCTCTG
Col 8a 1	CCTGGGTCAGCAAGIACCTC	TTGTTCCCCTCGTAAACTGG
Col 15	GCCTTCCTTGACATTGCTGAAGA	CTCCGTTGGACATAGAGAGGGTT
MMP-1	CCCAAAAGCGTGTGACAGTA	GAGCTCAACTTCCGGGTAGA
MMP-3	CAAGGAGGCAGGCAAGACAGC	GCCACGCACAGCAACAGTAGG
TIMP-1	TGGCTTCTGGCATCCTGTTGTTG	CGCTGGTATAAGGTGGTCTGGTTG
IGF-1	AGCTGGTGGATGCTCTTCAGTT	GAAGCAGCACTCATCCACGAT
TGF-IS	AAGGGCTACCACGCCAACTT	CCGGGTTGTGCTGGTTGTAC

Table 2

Gene Expression of Components of the Arachidonic Acid Cascade in Human NP Cells

Gene Symbol	Gene Title	Known Function	Basal (−TNFα)	Induced (+TNFα)
Cyclooxygenases				
PTGS1	Cyclooxygenase 1 (COX-1)	Constitutive COX. PTG biosynthesis	67.2 ± 25.3	51.3 ± 24.4
PTGS2	Cyclooxygenase 2 (COX-2)	Inducible COX. PTG biosynthesis	**206.3** ± 37.4	**401.3** ± 37.1
Isomerases				
PTGES	Prostaglandin E synthase	Microsomal glutathione-dependent PGE synthase. P53-induced gene	**413.1** ± 189.5	**1150.2** ± 454.3
PTGES2	Prostaglandin E synthase 2	Membrane-associated PGE synthase Converts PGH_2 to PGE_2	264.3 ± 81.1	304.1 ± 116.1
PTGES3	Prostaglandin E synthase 3	Constitutive cytosolic PGE synthase Chaperone for steroid receptor	8542 ± 2302	9988.9 ± 3535.3
PTGDS	Prostaglandin D2 synthase	Converts PGH_2 to PGD_2	177.7 ± 46.8	186.8 ± 47.7
AKR1C3 (PTGFS)	Prostaglandin F synthase	Converts PGH_2 to $PGF_{2\alpha}$	**730** ± 298	**1001** ± 392.6
PTGIS	Prostaglandin 12 synthase (prostacyclin synthase)	Converts PGH_2 to PGI_2	**1320** ± 215.5	**407.9** ± 16
PTG receptors				
PTGER1	Prostaglandin E receptor 1	One of four receptor for PGE_2	32.4 ± 10.2	26.8 ± 8.5
PTGER2	Prostaglandin E receptor 2	One of four receptor for PGE_2	**889.6** ± 160.6	**2278.5** ± 173
PTGER3	Prostaglandin E receptor 3	One of four receptor for PGE_2	25.2 ± 3.2	18.4 ± 4.5
PTGER4	Prostaglandin E receptor 4	One of four receptor for PGE_2	**417** ± 236	**752.9** ± 603.7
PTGFR	Prostaglandin F receptor	Receptor for $PGF_{2\alpha}$	**266.9** ± 73.7	**719.6** ± 118.9
PTGIR	Prostaglandin 12 receptor	Receptor for prostacyclin (PGI_2)	58.3 ± 10.7	67.2 ± 17.1

Microarray analysis of gene expression of components of the arachidonic acid cascade. RNAs from hNP cells exposed to 0 and 5 ng/ml TNF-α for 24 h were hybridized to Affymetrix GeneChip Human Genome U133 Plus 2.0 Arrays mRNA expression levels of genes involved in the arachidonic acid pathway were analyzed. Basal = untreated control. Values are mean ± SE of $n = 3$. Genes expressed highly at basal level or inducible by TNF-α are highlighted in bold face.