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Signaling by Mechanical Strain Involves Transcriptional Regulation of Proinflammatory Genes in Human Periodontal Ligament Cells In Vitro

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Abstract

Intracellular signals generated by mechanical strain profoundly affect the metabolic function of osteoblast-like periodontal ligament (PDL) cells, which reside between the tooth and alveolar bone. In response to applied mechanical forces, PDL cells synthesize bone-resorptive cytokines to induce bone resorption at sites exposed to compressive forces and deposit bone at sites exposed to tensile forces in an environment primed for catabolic processes. The intracellular mechanisms that regulate this bone remodeling remain unclear. Here, in an in vitro model system, we show that tensile strain is a critical determinant of PDL-cell metabolic functions. Equibiaxial tensile strain (TENS), when applied at low magnitudes, acts as a potent antagonist of interleukin (IL)-1 β actions and suppresses transcriptional regulation of multiple proinflammatory genes. This is evidenced by the fact that TENS at low magnitude: (i) inhibits recombinant human (rh)IL-1 β -dependent induction of cyclooxygenase-2 (COX-2) mRNA expression and production of prostaglandin estradiol (PGE₂); (ii) inhibits rhIL-1 β -dependent induction matrix metalloproteinase-1 (MMP-1) and MMP-3 synthesis by suppressing their mRNA expression; (iii) abrogates rhIL-1 β -induced suppression of tissue inhibitor of metalloproteinase-II (TIMP-II) expression; and (iv) reverses IL-1 β -dependent suppression of osteocalcin and alkaline phosphatase synthesis. Nevertheless, these actions of TENS were observed only in the presence of IL-1 β , as TENS alone failed to affect any of the aforementioned responses. The present findings are the first to show that intra-cellular signals generated by low-magnitude mechanical strain interfere with one or more critical step(s) in the signal transduction cascade of rhIL-1 β upstream of mRNA expression, while concurrently promoting the expression of osteogenic proteins in PDL cells.

Keywords

Bone; Mechanical tensile strain; Periodontal ligament (PDL) cells; Interleukin-1 β (IL- β); Osteogenesis

Introduction

Periodontal ligament (PDL) cells are highly specialized cells that reside between tooth and bone, and differentiate into cementoblasts to synthesize cementum of the teeth and osteoblasts to synthesize alveolar bone for the skeletal support of the tooth. In response to applied mechanical forces, osteoblast-like PDL cells perceive mechanical signals and respond to them via cellular events such as cell proliferation, differentiation, matrix catabolism, and matrix synthesis.^{6,9,16,24,25} These events, in turn, are controlled by sequential synthesis of cytokines and growth factors that regulate bone resorption at compression sites and bone synthesis at tension sites.^{9,14,16,23} It is well documented that compressive forces induce expression of proinflammatory mediators, and interleukin- β (IL-1 β) has been implicated as one of the major cytokines synthesized in response to compressive forces exerted on the PDL.^{4,13,14,16,23} IL-1 β stimulates prostaglandin estradiol (PGE₂) synthesis,^{9,16,19,23–25,30} Nitric oxide (NO) production,^{9,28,30} and secretion of proteases.^{7,10,19} Simultaneously, IL-1 β induces synthesis of matrix metalloproteinases (MMPs) to augment matrix degradation,^{2,12,18} and also inhibits synthesis of tissue inhibitor of metalloprotease-II (TIMP-II), a potent suppressor of MMP activity.² IL-1 β also downregulates the synthesis of osteogenic proteins such as osteocalcin and alkaline phosphatase. All of these events enhance catabolic activity and are involved in bone destruction.^{2,9,10,22–25} IL-1 β and PGE₂ are found at both tension and compression sides of the tooth.^{9,13,23–25} The presence of these bone-resorptive mediators in the PDL has raised the question as to how anabolic events like bone deposition occur at tension sites in an environment primed for catabolic processes.

Recent reports have demonstrated that cyclic equibiaxial tensile strain (TENS) acts on osteoblast-like cells in a dose-dependent manner. For example, we demonstrated that TENS of low magnitude (3%–6% elongation) inhibits IL-1 β -induced synthesis of proinflammatory cytokines such as IL-1 β , IL-6, and IL-8 in PDL cells.^{9,15,19} Similarly, TENS (1.8%–5% elongation) initiates anabolic events such as production of collagen type I, fibronectin, alkaline phosphatase, and transforming growth factor- β (TGF- β) in PDL cells,^{3,12,15,17} and also inhibits osteoclast formation in vitro.^{20,21} In contrast, TENS of higher magnitude (12.5%–18% elongation) induces PGE₂ and IL-1 β production in PDL cells in vitro.^{15,23–25,29} Based on the aforementioned observations, we speculated that intracellular signals generated by tensile strain antagonize the actions of proinflammatory signals and allow bone deposition despite the presence of inflammatory mediators in the PDL. Therefore, we examined the effects of TENS alone on PDL cells, as well as in the presence of IL-1 β , to mimic inflammatory conditions. We show that physical signals generated by mechanical strain are converted into explicit biochemical events that regulate metabolic functions of PDL cells. Furthermore, mechanical strain acts on the PDL cells in a dose-dependent manner. TENS at lower magnitude (1.8%–6% elongation) inhibits IL-1 β -induced synthesis of proinflammatory molecules and simultaneously augments synthesis of bone matrix-associated proteins. On the other hand, TENS at higher magnitude (12.5% elongation) is proinflammatory and induces synthesis of PGE₂ and IL-1 β ¹⁵ in PDL cells.

Materials and Methods

Reagents

Tissue culture media, calf serum (CS), fetal calf serum (FCS), and antibiotics were purchased from Gibco (Grand Island, NY); luminol, reflection autoradiographic film, and a PGE₂ radioimmunoassay kit were from NEN (Boston, MA); recombinant human IL- β (rhIL-1 β) was from Genetech (La Jolla, CA); collagen type I-coated Bioflex II culture plates were from Flexcell, Inc. (Hillsboro, NC); the RNA extraction kit was from Qiagen, Inc. (Santa Clara, CA); molecular biology reagents were from Perkin Elmer (Norwalk, CT); primers for polymerase chain reaction were from Biosynthesis, Inc. (Lewisville, TX); antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA); and all other reagents were from Sigma Chemical Co. (St. Louis, MO).

Isolation and Characterization of PDL Cells

Impacted healthy third molars removed for orthodontic reasons were obtained following approval from the internal review board, University of Pittsburgh. The root surfaces were washed, scraped, tissue minced, and cultured in tissue culture medium (TCM; containing basal Eagle's medium with 10% low endotoxin CS, 2 mmol/L glutamine, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 80 μ g/mL tylosin). The semiconfluent cultures were grown further in tylosin-free TCM and cloned by limiting dilutions.¹ PDL cell clones, designated PL-442 and PL-150 (from white females, ages 18 and 16 years), PL-484 (from white males, age 18 years), and PL-75 (Asian male, age 16 years), were characterized to assure their PDL (osteoblast-like) phenotype by the presence of high alkaline phosphatase activity, the constitutive expression of mRNA for TGF- β 1 and osteocalcin, and prostaglandin-induced cyclic AMP (cAMP) formation.^{1,3} Cells were used between passages 6 and 12. No significant differences in growth rate, AP activity, and expression of mRNA for TGF- β 1 and osteocalcin were observed between these passages.^{1,15}

Exposure of PDL Cells to Equibiaxial TENS and IL-1 β

PDL cells were seeded on collagen type I-coated Bioflex II plates (Flexcell International, Inc., Hillsboro, NC) at a rate of 5×10^5 /well. The confluent PDL-cell cultures (6–8 days old) were washed and incubated overnight in TCM without CS, but supplemented with SRM-1. To provide uniform radial and circumferential strain on the Bioflex plate membrane, the plates were placed on a loading station (located in a 5% CO₂ incubator). The loading station was connected to a Flexercell unit with a computer-assisted cyclic vacuum controller. The strain was calculated as: circumferential strain = $2\pi(\text{change in radius})/2\pi(\text{original radius}) = (\text{change in radius})/(\text{original radius}) = \text{radial strain}$. The magnitude of TENS was varied between 1.8% and 12.5% at 0.005 Hz to determine the effects of various magnitudes of TENS on PDL cells.^{15,28} PDL cells were divided into four groups: untreated and unstressed control cells; cells treated with TENS alone; cells treated with IL-1 β alone; and cells treated with TENS and IL-1 β .

PDL cells subjected to various magnitudes of TENS alone exhibited minimal cell deformation, negligible cell detachment, or cell death, as assessed by DNA fragmentation (data not shown).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

PDL cells washed with phosphate-buffered saline (PBS), were subjected to RNA extraction with a Qiagen RNA extraction kit.²⁸ A total of 0.5 µg of RNA was mixed with 1 µg oligo-dT in reverse transcription buffer and incubated for 10 min at room temperature. Thereafter, the reaction mixture was cooled on ice and incubated with 200 U of murine Moloney Leukemia virus (M-MLV) reverse transcriptase for 60 min at 37°C. The cDNA was amplified with 0.1 µg of specific primers in a reaction mixture containing 200 µmol/L deoxynucleotide triphosphates glycerol dehydephosphate dehydrogenase (dNTP), and 0.1 U of Taq-polymerase in PCR buffer. PCR was performed in a DNA thermal cycler (Perkin Elmer) for 30 cycles of 40 sec at 94°C, 40 sec at 62°C, and 60 sec at 72°C. The sequence of sense and antisense human primers used were as follows: GAPDH (548 bp): sense 5'GGTGAAGGTTCG-GAGTCAACGG3', and antisense 5'GGTCATGAGTCTTC-CACGAT3'; inducible nitric oxide synthase (iNOS) (243 bp): sense 5'CGGTGCTGTATTTCTTACGAGCGAAGAAGG3', antisense 5'GGTGCTGCTTGTTAGGAGGTCAAGTAAAGG-GC3'; cyclooxygenase-2 (COX-2; 283 bp): sense 5'TTCAAAT-GAGATTGTGGGAAAATTGCT3', antisense 5'AGATCATCTC-TGCCTGAGTATCTT3'; collagenase (403 bp): sense 5'CTGT-TCAGGGACAGAATGTGCT3', antisense 5'TTGGACTCACAC-CATGTGTT3'; stromelysin-1 (505 bp): sense 5'CCCTCCAGA-ACCTGGGAC3', antisense 5'ATAAAAGAACCCAAATTCT-TCAAAA3'; TIMP-I (339 bp): sense 5'TACTTCCACAGGTC-CCACAACC3', antisense 5'GGCTATCTGGGACCGCAGGGA-CTGCCA3'; TIMP-II (414 bp): sense 5'GTAGTGATCAGGGC-CAAG3', antisense 5'TTCTCTGTGACCCAGTCCAT3'; osteocalcin (294 bp): sense 5'ATGAGAGCCCTCACACTCCTC3', antisense 5'CGGGCCGTAGAAGCGCCGATA3'.

Nitrite Determination

Total NO production was determined as the nitrite concentration in culture medium by the use of the spectrophotometric assay, based on the Griess reaction.²⁸

PGE₂ Measurements

PGE₂ was measured at various time intervals in the culture supernatants of PDL cells by radioimmunoassay kits according to the manufacturer's recommended protocols.²⁸

Western Blot Analysis

After various treatments, PDL cells (3×10^6) were washed with ice-cold PBS, scraped from Bioflex II plates, and immediately lysed in ice-cold 200 µL cell lysis buffer (CLB; 20 mmol/L HEPES [pH 7.5], 150 mmol/L NaCl, 1% NP-40, and 1 mmol/L Na₃VO₄) containing ethylene-diamine tetraacetic acid (EDTA)-free complete protease inhibitor cocktail (BM; 1 mmol/L benzamidine, 0.4 mmol/L phenomethylsulfonylfluoride [PMSF], 1 mmol/L sodium metabisulfite, 10 µg/mL leupeptin, and 10 µg aprotinin; Sigma), and centrifuged at 16,000g for 10 min.²⁸ The supernatant was cleared twice with 50 µL of GammaBind G-Sepharose slurry (Pharmacia). Total protein in lysates was determined by the Brad-ford assay (Biorad) using BSA (0–100 µg) as a standard. Collagenase and TIMP synthesis were assessed in 50 µg of protein extracts by western blot analysis,²⁸ using goat

anti-MMP-1, anti-TIMP-I, or anti-TIMP-II as the primary antibodies; donkey anti-goat horseradish peroxidase (HRP) as secondary antibodies; and luminol as a chemiluminescent HRP substrate. The semiquantitative assessment of the luminescence in each band was performed by exposing the blots to reflection autoradiographic film, followed by semiquantitative analysis of the luminescent bands using a Biorad Fluor-S MultiImager (Biorad, Hercules, CA).

Statistical Analysis

All experiments were performed at least three times and on at least three cell lines (PL-442, PL-150, PL-75, or PL-484). In each case, photographic images from one representative experiment from a total of three is presented. The significance of differences between mean values of experimental and control groups was determined by analysis of variance (ANOVA).

Results

Effects of Various Magnitudes of TENS on IL-1 β -dependent PGE₂ Production in PDL Cells

Determination of the effects of various magnitudes of TENS on IL-1-dependent PGE₂ production revealed that IL-1 β -induced PGE₂ production was suppressed significantly ($p < 0.05$) by coexposure of PDL cells to low magnitudes of TENS (1.8%, 3%, or 6%). In these experiments, PDL cells exposed to TENS alone or untreated control cells did not exhibit PGE₂ production. On the contrary, higher magnitudes of TENS (10% or 12.5%) not only failed to suppress IL-1 β -induced PGE₂ production, but were inflammatory and augmented PGE₂ production to a similar extent or higher than that observed in cells exposed to IL-1 β alone (Figure 1A). Subsequently, to focus on the anti-inflammatory and anabolic properties of TENS, in further experiments we examined the effects of TENS of lower magnitude (6%).

TENS of Low Magnitude Suppresses PGE₂ Synthesis Over a Wide Range of rhIL-1 β Concentrations

Because applied mechanical forces cause accumulation of IL-1 β at concentrations between 0.5 and 20 ng/mL in the PDL, we sought to determine whether 6% TENS could inhibit PGE₂ production in the presence of higher IL-1 β concentrations (0, 0.1, 0.5, 1, 5, 10, or 25 ng/mL). As shown in Figure 1B, 1 ng/mL of IL-1 β was sufficient to induce maximal PGE₂ production in PDL cells, whereas 6% TENS markedly inhibited ($p < 0.05$) IL-1 β -dependent PGE₂ production up to 10 ng/mL rhIL-1 β .

TENS Suppresses Induction of Multiple IL-1 β -dependent Proinflammatory Genes

We next examined whether the aforementioned inhibition of PGE₂ synthesis is mediated through suppression of COX-2 mRNA expression. As evidenced by RT-PCR bands, 6% TENS inhibited IL-1 β -induced COX-2 mRNA expression, which was paralleled by a sustained and significant reduction in PGE₂ synthesis (Figure 2A,B).

Because TENS inhibited mRNA expression for COX-2, it raised the possibility that 6% TENS may also downregulate the induction of iNOS mRNA and NO production. Examination of four different PDL cell lines (PL-442, PL-150, PL-484, and PL-75) showed that PDL cells neither express iNOS mRNA (Figure 2A) nor produce NO in significant quantities in response to IL-1 β or TENS (data not shown).

IL-1 β induces synthesis of MMPs to initiate soft and hard tissue destruction; therefore, we next determined whether 6% TENS also inhibits matrix degradation by inhibiting the synthesis of MMPs. Examination of MMP-1 and MMP-3 revealed that 6% TENS significantly suppresses the IL-1 β -induced MMP-1 and MMP-3 mRNA expression within first 4 h, which was increasingly inhibited in a time-dependent manner over the ensuing 48 h (Figure 2A). This reduction in mRNA expression was also paralleled by inhibition of MMP-1 and MMP-3 synthesis (Figure 2C). MMP-1 or MMP-3 mRNA expression or synthesis was not observed in cells treated with 6% TENS alone or in untreated control cells.

TENS Abrogates IL-1 β -dependent Inhibition of Tissue Inhibitors of TIMP-II

We next examined whether 6% TENS also suppresses proinflammatory responses of IL-1 β by abrogating IL-1 β -induced inhibition of TIMP-I or TIMP-II synthesis. The densitometric analysis of the PCR products for TIMP-I revealed that exposure to IL-1 β neither inhibits TIMP-I mRNA expression nor its synthesis during the entire period of incubation (Figure 3A). Nevertheless, IL-1 β consistently and significantly ($p < 0.001$) inhibited the constitutive expression of TIMP-II mRNA over a period of 48 h. This inhibition of IL-1 β -induced TIMP-II induction was abrogated by coexposure of PDL cells to 6% TENS (Figure 3B). In fact, the semiquantitative western blot analysis of TIMP-II revealed that, in comparison to control cells, 6% TENS induced 2.6- and 4.2-fold increases in TIMP-II synthesis after 24 and 48 h, respectively (Figure 3C). The effects of 6% TENS were IL-1 β -dependent, as 6% TENS alone did not affect TIMP-II mRNA expression nor its synthesis (Figure 3B).

Signals Generated by TENS of Low Magnitude Are Reparative in Nature and Induce Proteins Involved in Bone Synthesis

One of the common events during inflammation is inhibition of the synthesis of osteogenic proteins, such as osteocalcin and alkaline phosphatase. To determine whether TENS, while inhibiting induction of multiple proinflammatory proteins, also acts as a reparative signal to induce osteogenic proteins, we examined the induction of osteocalcin in PDL cells. The semiquantitative densitometric analysis of the osteocalcin bands of RT-PCR products and protein bands on western blots revealed that exposure to IL-1 β inhibited constitutive osteocalcin gene induction, by inhibiting its mRNA expression and synthesis (Figure 4A,B). This IL-1 β -induced inhibition of osteocalcin expression was abrogated by 6% TENS. However, PDL cells treated with TENS alone did not exhibit increased induction of osteocalcin when compared with untreated control cells (Figure 4B).

Analysis of alkaline phosphatase activity in PDL cells revealed that IL-1 β significantly ($p < 0.05$) inhibited alkaline phosphatase activity, whereas coexposure of cells with 6% TENS abrogated this inhibition at all timepoints tested (Figure 4C). Interestingly, signals generated

by TENS alone were not sufficient to augment the expression alkaline phosphatase activity above those constitutively expressed in untreated control cells.

Discussion

The major finding of this study is that tensile strain of low magnitude acts as a potent antagonist of IL-1 β actions. This was demonstrated by the fact that 3%–6% TENS inhibited IL-1 β -dependent PGE₂ production. Focusing on the anti-inflammatory effects of TENS revealed that 6% TENS markedly inhibited IL-1 β -induced mRNA abundance of multiple proinflammatory proteins such as COX-II, MMP-1, and MMP-3. These findings suggest that TENS, as a potent downregulator of IL-1 β actions, acts at a critical step(s) in the signal transduction cascade of IL-1 β upstream of mRNA expression. IL-1 β is a major mediator of hard and soft tissue destruction in the PDL during inflammation. Therefore, by inhibiting the signal transduction pathway of IL-1 β , intracellular signals generated by TENS may be critical in inhibiting tissue destruction during inflammation.

It is important to note that TENS of low magnitude alone did not induce proinflammatory signals, but acted as an effective anti-inflammatory signal in the presence of IL-1 β . This is not surprising as a number of reports have recently shown that inhibitors of PGE₂ synthesis inhibit bone synthesis, implicating the requirement of PGE₂ in the initiation of bone repair.^{5,8,11,27} For example, Chambers et al.^{5,8} showed that, during the initial transduction of mechanical signals, the presence of PGE₂ is essential to induce an osteogenic response during bone formation in vivo. Similarly, a transient increase in COX-2 expression at the initial stages of bone adaptation has been observed by Chow et al.⁸ and Turner et al.²⁶ The present findings also suggest that the signals generated by physiologic or low levels of TENS may require activation of the proinflammatory signal transduction cascade to initiate osteoinduction.

The presence of another potent proinflammatory molecule, NO, is required for bone formation in osteoblast-like cells.^{5,8,11,27} This has been attributed mainly to the production of endothelial NOS (eNOS) in response to mechanical strain.³⁰ We observed that neither iNOS mRNA nor high levels of NO are induced by IL-1 β in PDL cells. However, we did not examine the effects of TENS on eNOS mRNA induction in the presence or absence of IL-1 β . Because eNOS mediated low levels of NO induction was not measurable by the Griess reaction in our studies, and thus the possibility of eNOS induction by TENS cannot be excluded. In this context, it is also important to note that, although exogenous NO potentiates bone formation, the effects of NO are secondary to PGE₂ as NO alone is not sufficient for osteoinduction,²⁶ raising the possibility that PGE₂ may be the major mediator of osteogenic responses in PDL cells.

In the present experiments we used IL-1 β as a proinflammatory molecule because it has been found in significant concentrations in the PDL at sites exposed to compressive forces as well as to tensile forces during tooth movement.^{9,16,23,24} The exact frequencies of mechanical strain exerted on PDL cells during tooth movement are not clear. Due to the fact that PDL cells are exposed to prolonged but variable frequencies of TENS, we used TENS at

0.005 Hz (200 sec/cycle), a relatively lower frequency than used in earlier studies.^{21,25} Whether TENS exerts its anti-inflammatory effects at higher frequencies remains unknown.

We observed that TENS exerts its actions on PDL cells in a dose-dependent manner. TENS at low magnitude (1.8%–6% elongation) generates potent anti-inflammatory signals, whereas TENS at high magnitude (10%–15% elongation) is an inflammatory signal and induces PGE₂ and IL-1 β ,¹⁵ both implicated in matrix degradation. Ample evidence exists to suggest that mechanical forces of high magnitude (12.5%–18%) evoke inflammatory responses in PDL cells that are characterized not only by the induction of IL-1 β in the periodontal tissue, but also by the presence of PGE₂.^{10,22,24,25} For example, the application of mechanical forces on teeth induces PGE₂ production in vivo,^{4,12} and IL-1 β and PGE₂ production in PDL cells in vitro.^{15,18,22,24,25} In vivo, PGE₂ production in response to mechanical stress has been shown to be associated with bone resorption, whereas the application of exogenous PGE₂ was shown to enhance matrix degradation and osteoclastic differentiation in response to applied mechanical forces.^{7,21–25} Our results concur with these findings in that the mechanical strain of higher magnitude (12.5%) exerts catabolic effects on PDL cells via sustained PGE₂ production. Moreover, we observed that 12.5% or higher magnitudes of TENS synergize with IL-1 β in inducing PGE₂ production, as well as IL-1 β induction.¹⁵ Whether signal transduction mechanisms of mechanical strain of higher magnitudes are mediated by pathways similar to those used by IL-1 β remains unclear. Nevertheless, one can deduce from these observations that the basis of extensive bone resorption observed in response to applied forces of high magnitude may be due to the synergistic effects of both proinflammatory mediators and mechanical stress on PDL cells.

Interestingly, anti-inflammatory actions at physiologic or low levels of TENS not only include inhibition of the synthesis of proinflammatory molecules, but also a significant hyperinduction of anti-inflammatory molecules that suppress matrix degradation. For example, IL-1 β inhibits TIMP-II synthesis in PDL cells, whereas TENS abrogates the IL-1 β -induced inhibition of TIMP-II synthesis. In fact, TENS, in the presence of IL-1 β , hyperinduced TIMP-II synthesis that was 2.6- and 4.2-fold greater than untreated control cells and IL-1 β -treated cells, respectively. This augmentation of TIMP-II synthesis could only be observed in the presence of IL-1 β . However, TENS alone was not able to augment TIMP-II synthesis in PDL cells.

In addition, TENS of low magnitude acts as an anabolic signal and induces synthesis of bone matrix-associated proteins. Treatment of PDL cells with IL-1 β led to the loss of their osteoblast-like phenotype, as evidenced by their failure to express high levels of alkaline phosphatase activity and osteocalcin.^{1,22,29} Our findings show that TENS of low magnitude alone did not induce osteocalcin or alkaline phosphate expression levels above those present in untreated control cells. Nevertheless, TENS in the presence of IL-1 β stimulated osteocalcin expression that was 1.2-fold greater than in untreated control cells, and 5.2-fold greater than in cells treated with IL-1 β . Similarly, TENS abrogated IL-1 β -induced inhibition of alkaline phosphatase synthesis in PDL cells. It is of interest that tensile forces generated by low levels of TENS have also been shown to inhibit osteoclastogenesis and expression of osteoclast-activating factor in murine stromal cells, demonstrating another role of TENS in osteogenesis.^{20,21} Thus, it is tempting to speculate that, while inhibiting proinflammatory

signals, the mechanical signals of low magnitude integrate the metabolic signals within the PDL cells in a manner that promotes osteogenesis in the presence of inflammatory signals.

Extrapolation of the aforementioned in vitro actions of TENS on PDL cells to an in vivo situation suggests that: (i) osteoblast-like PDL cells remodel alveolar bone according to their mechanical environment, that is, signals generated by low or physiologic levels of tensile strain initiate repair by suppressing the actions of proinflammatory mediators, while initiating simultaneous induction of osteogenic molecules; and (ii) signals generated by mechanical tensile strain of low magnitude do not initiate bone formation in the presence of a proinflammatory signal. It appears that the signals generated by a low magnitude of strain interfere with transcriptional regulation of multiple proinflammatory genes activated or suppressed by IL-1 β , that is, it disrupts or downregulates one or more critical step(s) in the signal transduction cascade of IL-1 β upstream of mRNA transcription for proinflammatory genes. Thus, via suppressing PGE₂ and MMP production, as well as inducing anabolic proteins, it may inhibit bone resorption and channel cellular events toward bone formation at the sites exposed to tensile strain during tooth movement. On the other hand, nonphysiologic higher magnitudes of TENS provoke proinflammatory signals, which may consequently induce bone resorption, frequently observed at the tension sites in response to a higher magnitude of applied mechanical forces.^{4,6,23,29} In both of these processes, molecules of the proinflammatory signal transduction pathways may act as intermediaries for the mechanotransduction pathways to induce synthesis of bone. Whether TENS specifically inhibits IL-1 β actions or whether it can counteract actions of other inflammatory mediators like TNF- α , PGE₂, or endotoxins found in inflamed PDL, has yet to be determined.

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Figure 1.

(A) Effect of various magnitudes of TENS on IL-1β-dependent PGE₂ production. PDL cells were incubated with IL-1β (1 ng/mL) and simultaneously exposed to 0%, 3%, 6%, 10%, or 12.5% TENS for 24 h. (B) Effect of TENS on PGE₂ production in response to various concentrations of rhIL-1β. PDL cells were treated with 0, 0.1, 0.5, 1, 5, 10, or 25 ng/mL of rhIL-1β for 24 h with or without simultaneous exposure to 6% TENS for 24 h. PGE₂ accumulation in the culture supernatants was assessed by radioimmunoassay. Data represent mean and SEM of triplicate values in all experiments. **p* < 0.05 vs. cells treated with rhIL-1β alone; ✱*p* < 0.05 vs. untreated control cells.

Figure 2. Effect of TENS (6%) on the IL-1 β -induced induction of proinflammatory genes. (A) RT-PCR analysis for COX-2, iNOS, MMP-1, and MMP-3 mRNA expression in PDL cells either untreated, or subjected to 1 ng/mL rhIL-1 β , 6% TENS, or rhIL-1 β and TENS. (B) Effect of TENS (6%) on the IL-1 β -induced PGE₂ accumulation in the culture supernatants of cells exposed to various treatment regimens as described in (A). (C) Semiquantitative densitometric analysis of protein bands in western blot analysis showing relative expression of MMP-1 and MMP-3 in PDL cells subjected to treatment regimens shown in (B). The data represent percent of total IL-1 β -induced PGE₂, MMP-1, and MMP-3 synthesized in the presence of 6% TENS. Data represent one of three separate experiments in each graph. Amplification of GAPDH mRNA was used to assure equal input in all lanes. Bars in (B)–(D) represent means and SEM of triplicate values. * p < 0.05 vs. cells treated with IL-1 β alone.

Figure 3.

Effect of 6% TENS on rhIL-1 β -dependent inhibition of TIMPs. (A) RT-PCR analysis of mRNA for TIMP-I and TIMP-II in PDL cells either untreated or exposed to 1 ng/mL rhIL-1 β , 6% TENS, or rhIL-1 β and 6% TENS. (B) Western blot analysis showing TIMP-II synthesis in cell extracts (50 μ g/lane) subjected to treatment regimens described in (A) for 24 or 48 h. (C) Semiquantitative densitometric analysis of TIMP-II bands in (B) showing hyperinduction of TIMP-II by 6% TENS in the presence of IL-1 β . Each lane received a total of 100 μ g protein. * p < 0.05 vs. cells treated with IL-1 β alone.

Figure 4.

Effect of 6% TENS on IL-1 β -induced inhibition of osteocalcin and alkaline phosphatase synthesis. (A) RT-PCR analysis of mRNA for osteocalcin in PDL cells exposed to various treatments regimens for 4, 24, or 48 h. Densitometric analysis of western blots showing osteocalcin synthesis (B), and alkaline phosphatase activity (C), in cells subjected to indicated treatment regimens for 24 or 48 h. Data represent mean and SEM of triplicate values. * $p < 0.05$ vs. cells treated with IL-1 β alone.