BIOMECHANICAL SIGNALS BLOCK I-kB ACTIVATION TO INHIBIT NF-κB-MEDIATED PROINFLAMMATORY GENE TRASCRIPTION IN ARTICULAR CHONDROCYTES

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INTRODUCTION

The beneficial effects of continuous passive motion on arthritic knees are well recognized, and signals generated by mechanical strain are shown to inhibit inflammation of joints afflicted with antigen-induced arthiritis (1). The anti-inflammatory effects of biomechanical signals are mediated by the inhibition of the nuclear translocation of NF-κB *in vitro* (2), which subsequently leads to a significant inhibition of the transcription and translation of pro-inflammatory genes. In this study we examined the target molecules in the NF-kB pathway that are responsible for the inhibition of NF-κB nuclear translocation in a pro-inflammatory environment.

METHODS

Cell culture: Chondrocytes were obtained from the articular cartilage from the knees of 10-12-week-old Sprague-Dawley rats. Cells were released from the cartilage by enzymatic digestion, cultured in TCM [Ham/F12, 10% FBS, 10 μg/ml penicillin and 100 U/ml streptomycin, and 1% L-glutamine], and used during the first 3 passages. During these passages the chondrocytic phenotype (aggrecan, biglycan, versican and collagen type II) was found to be stable.

Application of CTS: Cells (7 X 10^4/well) were grown for 5-6 days on collagen I-coated BioFlex-I culture plates to 80% confluence and subjected to cyclic tensile strain (CTS) in a Flexcell loading station (Flexcell Int, NC) at a magnitude of 3% and frequency of 0.25 Hz. Four different treatment regimens were assigned: i) untreated controls, ii) cells treated with recombinant human IL-1β, iii) cells treated with CTS, iv) cells treated with CTS and with rhIL-1β. The cells were harvested following a 10, 30, 60 and 90 minutes exposure to all four regimens.

Real time PCR: PCR was performed on a Biorad iCycler iQ (Biorad, CA). Taqman primers and probes were used and the data was analyzed using the comparative threshold cycle (CT) method.

Western Blot analysis: The blots were probed with anti-IκB-α, anti-IκB-β (Santa Cruz Biotech, CA), and phospho-IκBα Ser32, and Ser 36, phospho-NF-κB p65 Ser536, and Ser276 antibodies. The binding of primary antibodies was revealed with a horseradish peroxidase (HRP)–conjugated secondary antibodies. The bands were visualized with Western Lightening chemiluminescence reagent used as a substrate for HRP. The blots were exposed to Blue Lite Autorad Film and assessed by densitometric analysis using Kodak Image Station 1000, and image J software

Immunofluorescence: Activation of NF-κB, IkB-α and IkB-β was assessed by immunofluorescence using rabbit anti-NF-κB p65, anti-IκB-α, or anti-IκB-β IgG as primary antibodies and Cy3-conjugated goat anti-rabbit IgG as secondary antibody. Phalloidin-FITC was used as a counter-stain to visualize F-actin. The stained cells were visualized under an epifluorescence microscope (Zeiss Axioimage), and densitometrically analyzed by Zeiss Axiovision software (Carl Zeiss, Germany).

Statistical analysis: All experiments were done three times, and means ± S.E.M. were calculated. One-Way ANOVA and the post hoc multiple comparison Dunnett's test were applied to determine whether significant differences exist between the groups. Values of $p \leq 0.05$ were considered to be significant.

RESULTS

1) IL-1β induced a rapid nuclear translocation of NF-κB at all the time points tested, whereas CTS inhibited this IL-1β induced nuclear translocation at 30, 60 and 90 minutes (Fig.1). 2) Inhibition of NF-kB nuclear translocation by CTS was paralleled by a significant suppression of more than 90% of the IL-1β-induced iNOS mRNA expression during the initial 90 minutes. 3) CTS blocked mRNA expression for I-κBα that is upregulated by the treatment of chondrocytes with IL-1β at 10, 30, 60 and 90 minutes. Examination of the IκB-α protein by Western blots and immunofluoresence revealed that IL-1β induced rapid degradation of IκB-α at 10 and 30 minutes, accompanied by its resynthesis at 60 and 90 minutes. Exposure to CTS resulted in an inhibition of IκB-α degradation at 10 and 30 minutes and an inhibition of resynthesis at 60 and 90 minutes. 4) IL-1β significantly induced the degradation of I-κBβ protein synthesis at all the time points tested and this degradation was inhibited by CTS at 30, 60 and 90 minutes. 4) Examination of the NF-κB dependent genes IL-R1, IL-R2, TRAF-1 and TRAF-2 revealed that IL-1β markedly increased the expression of these genes, while CTS significantly downregulated their expression.

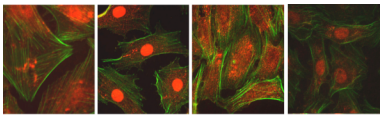

Fig 1. Inhibition of IL-1β induced NF-κB nuclear translocation by CTS at 30 minutes.

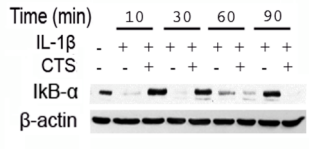

Fig 2. Regulation of IκB-α protein synthesis by CTS. CTS inhibits IL-1β induced degradation at 10 and 30 min and inhibits resynthesis at 60 and 90 min.

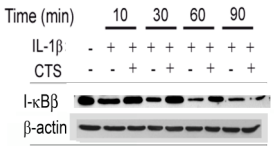

Fig 3. IL-1β induces degradation of IκB-β protein synthesis at all Time points, while CTS inhibits this IL-1β induced degradation.

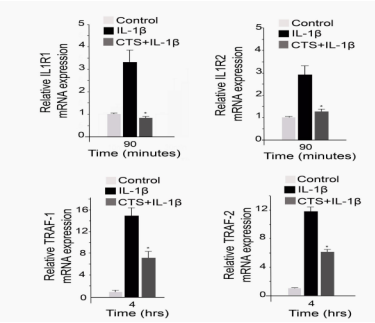

Fig 4. Inhibition of IL-1β dependent genes like IL-R1, IL-R2, TRAF-1 and TRAF-2 by CTS at 90 minutes. IL-1β significantly increases the expression of these genes as compared to control cells.

DISCUSSION

The results demonstrate that mechanical signals inhibit IL-1β induced inflammatory gene transcription by regulating the early events upstream of NF-κB nuclear translocation. Mechanical signals inhibit IL-1-induced inflammatory responses by both, blocking the activation of inhibitors of NF-κB, I-κBa, I-κBβ, and thus NF-κB. Furthermore, inhibition of nuclear translocation of NF-κB results in the Inhibition of NF-κB, I-κBα and I-κBβ mRNA expression and synthesis. Thus, mechanical signals regulate I-κBα in a time dependent manner causing inhibition of IL-1-induced I-κBα degradation at earlier time points and failure to inhibit resynthesis at later time points. Thus, by blocking NF-κB pathway at the site of I-κB activation, mechanical signals inhibit proinflammatory effects of IL-1β and help in avoiding tissue degradation induced by proinflammatory genes. [This study was supported by grants from NIH AT00646, AR04878, NIDCR DE15399]

References:

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