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Biomechanical strain regulates TNFR2 but not TNFR1 in TMJ cells

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Abstract

We sought to examine whether cyclic tensile strain (CTS) regulates the gene expression of tumor necrosis factor (TNF)-alpha, its receptors TNFR1 and TNFR2, and inducible nitric oxide synthase (iNOS) under inflammatory conditions, and whether these effects of CTS are sustained. Rat temporomandibular joint disc cells (TDC) were exposed to CTS in the presence or absence of interleukin (IL)-1beta for 4 and 24 h. Cells were also stimulated with IL-1beta for 24 h while being subjected to CTS only for the initial 1, 2, 4, 8, and 12 h or the entire 24 h incubation time. Furthermore, cells were incubated with IL-1beta for 24, 36, or 48 h while being exposed to CTS only for the initial 8 h. Gene expression of TNF-alpha, its receptors, and iNOS was analyzed by RT–PCR, whereas protein synthesis was determined by ELISA for TNF-alpha, immunofluorescence for TNFRs, and Griess reaction for nitric oxide. CTS inhibited the IL-1beta-stimulated synthesis of TNF-alpha, TNFR2, and iNOS. TNFR1 was constitutively expressed but not regulated by IL-1beta or CTS. Application of CTS for only 1 or 2 h during a 24 h incubation with IL-1beta was sufficient to inhibit IL-1beta-induced upregulation of TNF-alpha, TNFR2, and iNOS. However, for maximal inhibition of these genes a longer exposure of CTS was required. These findings are the first to show that biomechanical signals regulate the expression of TNFR2 but not TNFR1 under inflammatory conditions. Furthermore, the antiinflammatory effects of biomechanical signals on TDC are maintained for prolonged periods of time but are transient.

Keywords

TMJ; TNFR; TNF; INOS; Biomechanical strain

1. Introduction

Arthritis, an inflammatory disorder that is associated with the loss of structure and function of the joint, often affects the temporomandibular joint (TMJ) (Atsu and Ayhan-Ardic, 2006; Delgado-Molina et al., 1997). The TMJ, which is formed by the condyle of the mandible with the glenoid fossa and articular eminence of the temporal bone, also includes the fibrocartilaginous disc. The TMJ disc situated between the condyle and fossa-eminence is critical for load distribution and shock absorption (Detamore and Athanasiou, 2003a).

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Cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α as well as other inflammatory mediators such as nitric oxide (NO) play an important role in arthritic diseases by initiating and perpetuating the inflammatory process, causing pain and mediating destruction of cartilage and bone (Fermor et al., 2004; Fernandes et al., 2002; LeGrand et al., 2001; McDevitt and Miller, 1989). TMJ disc cells (TDC) are mechanosensitive and respond to physiological levels of mechanical signals by synthesis of collagen, proteoglycans, and glycosaminoglycans to fulfill their function as shock absorber (Detamore and Athanasiou, 2003b; Tanaka and van Eijden, 2003). However, in an inflammatory environment these cells change their phenotype and act as accessory inflammatory cells participating in the inflammation process by synthesizing cytokines, prostaglandins, and NO (Agarwal et al., 2001).

Several treatment modalities that target TNF-α, a key player in the pathogenesis of arthritis, are currently applied to inhibit joint inflammation as well as destruction of cartilage and bone. However, these treatment modalities often provide only partial relief of pain and stiffness. Additionally, they have side effects which limit their usefulness (Keystone and Kavanaugh, 2005; O'Dell, 2004; Scheinfeld, 2005). A promising alternative approach to suppress inflammation and catabolic processes in arthritis is exercise or mechanical loading of inflamed joints. It has been demonstrated that articular cartilage is better preserved in arthritic knees treated with continuous passive motion (CPM) than with immobilization (Kim et al., 1995). Clinical applications of CPM in various joints have also produced very satisfactory results (Israel and Syrop, 1997; Johnson and Eastwood, 1992; Salter, 1996). However, it has yet to be elucidated whether constant motion over long periods of time or precise durations of such therapeutic interventions are required to achieve optimal results for patients. Furthermore, it is yet unknown whether the beneficial effects of CPM persist following the removal of the biomechanical stimulus, which has important implications for clinical applications.

A number of in vitro studies have demonstrated that biomechanical strain at physiological levels can be reparative, anticatabolic, and antiinflammatory (Agarwal et al., 2001; Chowdhury et al., 2003, 2006; Deschner et al., 2005; Gassner et al., 2000). TNF-α takes an active role in fibrocartilage destruction during inflammation of the TMJ (Fu et al., 1995). The actions of TNF-α are mediated as well as regulated by its receptors, TNFR1 and TNFR2 (Campbell et al., 2003).

Hydrostatic pressure at excessively high levels has been shown to induce TNF-α expression in a chondrosarcoma cell line (Takahashi et al., 1998). Furthermore, pressure also affects the expression of TNFR1 in chondrocytes (Westacott et al., 2002). However, whether cyclic tension, another critical aspect of joint load, regulates the gene expression of TNF-α and its receptors in fibrocartilaginous cells of the TMJ has yet to be determined.

In this in vitro study we sought to determine whether biomechanical signals regulate the gene expression of TNF-α, TNFR1, TNFR2, and iNOS under inflammatory conditions. Furthermore, we studied whether the possible regulatory effects of biomechanical strain on these molecules are sustained. This study demonstrates that biomechanical signals under inflammatory conditions inhibit TNF-α, TNFR2, and iNOS but not TNFR1 in TDC.

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Furthermore, this study provides evidence that the antiinflammatory effects of biomechanical signals are maintained for several hours after their removal, beyond which gene expression for TNF-α, TNFR2, and iNOS resumes again.

2. Material and methods

2.1. Cell cultures

Cells were harvested from TMJ discs of 10–12-week old Sprague Dawley rats (Harlan, IN). Discs were cleaned, minced and transferred onto macroporous filters (Spectrum Laboratories, CA) placed in a two-compartment digestion chamber. After incubating with 0.2% trypsin for 10 min and 0.15% collagenase I (Worthington, NJ) for 2 h, cells were centrifuged and the pellet was resuspended in DMEM/F12 (Mediatech, VA) supplemented with 1% Penicillin/Streptomycin (Mediatech), 1% L-Glutamine (Invitrogen, NY), and 10% FCS (Hyclone®, UT). Cells were used between the third and fourth passage. The phenotype of TDC was confirmed by the synthesis of collagen type I, aggrecan, biglycan, and versican (Fig. 1A).

2.2. Application of cyclic tensile strain

TDC (5×10^5/well) seeded on collagen type I-coated BioFlex® culture plates (Flexcell® International, NC) were grown to 70%–80% confluence (4–5 days) in 5% CO_2 and 37 °C. One day before experiment, the FCS concentration was decreased to 1%. Cells were subjected to equibiaxial cyclic tensile strain (CTS) by placing BioFlex® culture plates on a loading platform equipped with round loading posts in a FX-4000 T Flexercell Tension System (Flexcell International) for 1, 2, 4, 8, 12, or 24 h. Cells were subjected to CTS at 20% and 0.05 Hz in the presence or absence of rhIL-1β (1 ng/ml; Calbiochem, CA).

2.3. Treatment of cells

To determine whether mechanical signals regulate TNF-α, TNFR1, TNFR2, and iNOS under inflammatory conditions, TDC were subjected to CTS in the presence or absence of IL-1β for 4 and 24 h. To investigate whether such a possible regulatory effect of mechanical loading is sustained, cells were stimulated with IL-1β for 24 h while being subjected to CTS either only for the initial 1, 2, 4, 8, and 12 h or the entire 24 h-incubation time. In addition, cells were incubated with IL-1β for 24, 36, and 48 h, while CTS was applied only for the initial 8 h. Unstretched cells in the presence or absence of IL-1β served as controls.

2.4. Reverse transcriptase–polymerase chain reaction

To verify the fibrocartilaginous phenotype of TDC the gene expression of collagen type I, aggrecan, biglycan, and versican was analyzed by endpoint reverse transcriptase–polymerase chain reaction (RT–PCR). RNA was extracted according to the manufacturer's recommended protocols by an RNA extraction kit (Qiagen, CA). A total of 1.0 μg of RNA was reverse transcribed with 200 U of M-MLV reverse transcriptase (Invitrogen, CA) at 42 °C for 25 min followed by 65 °C for 5 min. The cDNA was amplified with 0.1 μg of specific primers (Table 1) in a reaction mixture (PCR supermix, Invitrogen) containing Taq DNA polymerase, Tris-HCl, KCl, $MgCl_2$, and dNTPs. Amplification was carried out for 30 cycles of 45 s at 94 °C, 45 s at 59 °C, and 60 s at 72 °C by Mastercycler Gradient

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(Eppendorf, Germany). The bands of ethidium bromide-stained PCR products on agarose gels were visualized by a Kodak Image Station 1000 (Eastman Kodak Company, NY).

To study the gene expression of TNF-α, TNFR1, TNFR2, and iNOS in TDC real-time RT–PCR was performed using an iCycler iQ detection system (Biorad, CA). Specific primers and probes were designed with Primer Express® (Table 2). A 1 μl of cDNA as a template was amplified in a 25 μl reaction mixture containing 12.5 μl TaqMan® 2 × PCR Mastermix (Applied Biosystems, CA), 300 nM of each primer, 250 nM probe, and deionized water. The mixture was heated initially at 95 °C for 3 min and then followed by 40 cycles with denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. GAPDH was used as a house-keeping gene. To analyze the data, the comparative threshold cycle (CT) method was applied (Deschner et al., 2005; Giulietti et al, 2001; Livak and Schmittgen, 2001).

2.5. Immunofluorescence

The effect of CTS on TNFR1 and TNFR2 protein synthesis was analyzed by immunofluorescence. Cells attached to the flexible bottom of the BioFlex® plates were fixed with cold (–20 °C) 100% methanol for 5 min, washed with PBS, and blocked with Protein Blocking Agent (Thermo Electron, PA) and normal donkey serum (Santa Cruz, CA). Goat polyclonal anti-rat TNFR1 and TNFR2 (Santa Cruz) antibodies in combination with fluorescein-conjugated donkey anti-goat IgG (Jackson Immuno, PA) were applied. Cells were observed under 20× or 40× objectives, with an Axioplan 2 imaging microscope (Zeiss, NY). The images were captured with an AxioCam HR camera and Axiovision 4.1 capturing software (Zeiss).

2.6. Elisa

TNF-α levels in culture supernatants were measured by enzyme linked immunosorbent assay (ELISA) using the Quantikine rat TNF-α kit (R&D Systems, MN) according to the manufacturer's protocol. The optical density of each well was determined at 450 nm within 30 min, using a VICTOR3™ plate reader (Perkin-Elmer).

2.7. Measurement of nitric oxide

The presence of NO in the culture supernatants was determined by measuring the amount of nitrite, a metabolic product of NO, using the Griess reaction (Green et al., 1982).

2.8. Statistical analysis

The SPSS 13.0 software (SPSS Inc., IL) was used for statistical analysis. For quantitative analysis of the mRNA expression, mean values and standard errors of the mean (SEM) were calculated ($n = 6$). A statistical analysis was performed for each experiment: to determine whether significant differences exist between the groups (untreated cells, IL-1β-stimulated cells, cells subjected to CTS, and IL-1β-stimulated cells simultaneously subjected to CTS), one-way ANOVA and the post-hoc multiple comparison Tukey test were applied. To compare unstretched IL-1β-treated cells with different groups of stretched IL-1β-treated cells, one-way ANOVA and the post-hoc multiple comparison Dunnett test were used.

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Differences were regarded as statistically significant at values of $p<0.05$. Each experiment was performed at least three times.

3. Results

3.1. CTS inhibits TNF-α, TNFR2, and iNOS but not TNFR1 under inflammatory conditions

TDC constitutively expressed TNFR1 and TNFR2, whereas TNF-α and iNOS were not spontaneously expressed (Fig. 1B–E). IL-1β, which was used to mimic an inflammatory environment, induced a strong upregulation of TNF-α and iNOS following 4 and 24 h of IL-1β incubation (Fig. 1B and C). IL-1β also significantly increased the constitutive gene expression of TNFR2 but not TNFR1 (Fig. 1D and E). CTS when applied simultaneously to IL-1β-treated cells inhibited the IL-1β-stimulated mRNA expression of TNF-α, TNFR2, and iNOS by 78%, 20%, and 56%, respectively, following 4 h of strain and by 93%, 49%, and 96%, respectively, following 24 h of strain (Fig. 1B, C, and E). By contrast, the gene expression of TNFR1 in IL-1β-treated cells was not affected by CTS (Fig. 1D). CTS in the absence of IL-1β did not significantly regulate the mRNA expression of these molecules (Fig. 1B–E). TNF-α and NO levels in culture supernatants at 24 h reflected the findings for TNF-α and iNOS observed at transcriptional level (Fig. 1F and G). The regulatory effect of CTS on the gene expression of TNFR2 under inflammatory conditions was also reflected at protein level, as evidenced by immunofluorescence following 24 h of strain. Both TNFR1 and TNFR2 were spontaneously produced but only TNFR2 protein synthesis was stimulated by IL-1β, and this stimulation was abolished when CTS was simultaneously applied to IL-1β-treated cells (Fig. 1H). Although the response of TDC to IL-1β in the presence or absence of CTS slightly varied between experiments the pattern of cell response was the same in all replicates.

3.2. The inhibitory effects of CTS on TNF-α, TNFR2, and iNOS under inflammatory conditions are maintained for prolonged periods of time following the removal of mechanical stimulation

Next, we sought to determine whether the antiinflammatory effect of CTS, i.e. the downregulation of the IL-1β-stimulated gene expression of TNF-α, TNFR2, and iNOS persists. TDC were incubated with IL-1β for 24 h while being subjected to CTS either only for the initial 1, 2, 4, 8, and 12 h or the entire 24 h-incubation time. Real-time PCR revealed that exposure of cells to CTS for only 1 h was sufficient to significantly reduce the IL-1β-stimulated mRNA expression for TNF-α when examined 23 h later (Fig. 2A). CTS for 1 and 2 h inhibited the IL-1β-induced TNF-α expression by 44% and 51%, respectively (Fig. 2A). Exposure of cells to CTS for 4, 8, 12, or 24 h suppressed the IL-1β-induced TNF-α gene expression by 90% or more, as measured by real-time PCR at the end of the 24 h-incubation period (Fig. 2A). Measurement of TNF-α protein in the culture supernatants confirmed these findings at the protein level (Fig. 2B).

Subsequently, we investigated whether the inhibitory effect of CTS on the IL-1β-stimulated TNFR2 expression persists after removal of CTS. Unlike TNF-α, exposure of cells to CTS for only 1 h failed to significantly suppress the IL-1β-upregulated mRNA expression for TNFR2 when measured 23 h later. However, CTS applied to IL-1β-treated cells for 2 h was

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sufficient to decrease the TNFR2 expression by approximately 20%. The suppressive effect of CTS was even more pronounced when cells were exposed to mechanical signals for 8 and 12 h or the entire 24 h-incubation time (Fig. 2C). The gene expression data for TNFR2 were confirmed at protein level by immunofluorescence (Fig. 2D).

Finally, we examined whether the CTS effects on the stimulated iNOS expression are maintained. Similar to TNF-α, exposure of CTS to cells for only 1 h was sufficient to significantly inhibit the IL-1β-induced iNOS expression. Application of CTS for 1 or 2 h suppressed the iNOS expression in IL-1β-treated cells by approximately 60% and longer exposure times were even more effective in downregulating the iNOS gene expression (Fig. 2E). Measurement of NO in the culture supernatants of cells exposed to CTS for various time intervals during the entire 24 h-incubation with IL-1β confirmed at protein level that the antiinflammatory effects of CTS were maintained (Fig. 2F). In all replicates, the pattern of cell response was identical.

Since CTS applied only for the initial 8 h during the entire 24 h-incubation with IL-1β had already a profound suppressive effect, we studied in the next series of experiments how long this effect would persist. Cells were incubated with IL-1β for 24, 36, and 48 h while CTS was applied only for the initial 8 h. For TNF-α and TNFR2, the IL-1β-stimulated gene expression of these molecules was suppressed at 24 h, as expected (Fig. 3A and B). However, exposure of cells to CTS for only 8 h was insufficient to significantly suppress the IL-1β-stimulated TNF-α and TNFR2 expression at 36 or 48 h, as analyzed by real-time PCR (Fig. 3A and B). A significant inhibition of the IL-1β-induced TNF-α and TNFR2 by CTS was also not observed at protein level at 36 and 48 h (data not shown). By contrast, the IL-1β-induced iNOS expression was still suppressed by 38% at 36 h (Fig. 3C). Like TNF-α and TNFR2, CTS applied for the initial 8 h was not sufficient to significantly suppress the IL-1β-induced iNOS gene expression at 48 h (Fig. 3C). NO levels in the culture supernatants reflected the findings for iNOS at transcriptional level. Exposure of cells to CTS for 8 h significantly suppressed the IL-1β-stimulated NO synthesis at 24 and 36 h (Fig. 3D). In addition, the IL-1β-induced NO production was also significantly decreased by CTS at 48 h but this inhibition was only 20% compared to 33% at 36 h and 77% at 24 h (Fig. 3D). This pattern of cell response was the same in all replicates.

4. Discussion

This study demonstrates the regulation of TNF-α its receptors, and iNOS by biomechanical signals in TMJ disc cells exposed to an inflammatory environment. Furthermore, we provide original evidence that these antiinflammatory effects of biomechanical signals persist for prolonged periods of time after their removal, beyond which gene expression for TNF-α, TNFR2, and iNOS resumes again.

TNF-α plays a critical role in initiating and perpetuating inflammation and matrix degradation in arthritis by regulating synthesis of other proinflammatory mediators, cell adhesion molecules, proliferation, and apoptosis. Its crucial pathogenetic role is also emphasized by the efficacy of anti-TNF-α-strategies in rheumatoid arthritis (Campbell et al., 2003). TNF-α, either membrane (m) bound or soluble (s), binds to two receptors, TNFR1

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and TNFR2 (Carpentier et al., 2004; Holtmann and Neurath, 2004). Although both receptors were coexpressed in TDC, only TNFR2 was regulated by IL-1β or biomechanical strain, suggesting that TNFR2 is much more inducible than TNFR1. Therefore, it is possible that the ratio TNFR1:TNFR2 may alter and control TNF-α responses under inflammatory conditions (Carpentier et al., 2004). TNFR1 binds sTNF-α with a much higher affinity than TNFR2 and has therefore been considered the major mediator of TNF-α actions (Grell et al., 1995). More recently, it has been demonstrated that TNFR2 is preferentially activated by mTNF-α in a paracrine and/or autocrine manner, which implies that the mTNF-α/TNFR2 system might play an important immunoregulatory role at the local level in arthritic joints (Haas et al., 1999; Holtmann and Neurath, 2004). Thus, the relative contribution of TNFR2 should not be underestimated. Our finding that CTS inhibited the synthesis of TNFR2 in stimulated cells suggests that CTS mediates its antiinflammatory actions not only by downregulating TNF-α but also its receptor TNFR2. The functional activity of TNFR2 is also controlled by shedding. TNFR shedding results in a soluble receptor consisting of only the extracellular component of the membrane bound receptor. Soluble receptors can function as antagonists but also as agonists by prolonging the half-life of TNF-α or facilitating the TNF-α interaction (Carpentier et al., 2004). However, a reduction in TNF-α and TNFR2 expression suggests that antiinflammatory effects of biomechanical signals may prevail.

In our study, IL-1β was used to mimic an inflammatory cell environment since this cytokine is a key player in inflammatory diseases and increased in synovial fluid of arthritic joints (Kay and Calabrese, 2004). In addition to TNF-α and TNFR2, IL-1β also induced synthesis of iNOS and its product NO, another key player in arthritis. It can augment vascular permeability in inflamed joints, generate destructive free radicals, induce inflammatory mediators, activate matrix metalloproteinases and cell apoptosis (Abramson et al., 2001; Miyasaka and Hirata, 1997). This study demonstrates that biomechanical strain inhibits the upregulation of NO synthesis in cells exposed to inflammation, which is in accordance to our previous findings in articular cells from rabbits (Agarwal et al., 2001; Long et al., 2001; Xu et al., 2000, Gassner et al., 1999). More importantly, our findings also underline that the antiinflammatory effects of biomechanical signals are not limited to only one inflammatory mediator of arthritis.

Our data showed that the antiinflammatory effects of CTS are maintained for prolonged periods of time following the removal of mechanical stimulation despite an inflammatory environment. However, this study also revealed that TNF-α, TNFR2, and iNOS reexpress following a defined period of rest in a proinflammatory environment, indicating that the antiinflammatory effects of biomechanical strain are transient.

Furthermore, the extent of the antiinflammatory effects was dependent on how long cells were exposed to CTS. For example, application of CTS for only 1 or 2 h caused a significant inhibition of the upregulated expression for TNF-α, TNFR2, and iNOS, whereas for maximal inhibition of these genes a longer exposure of CTS was required. These results may have important implications for clinical applications and suggest that mechanical loading of the joint or exercise for only a limited time might be sufficient to suppress inflammation and promote healing.

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Our data reveal that the suppressive effect of CTS is earlier and stronger for TNF-α and iNOS as compared to TNFR2, and differences were also obvious between TNF-α and iNOS. The reason for these findings is as yet unknown. However, the promoter region for TNF-α, TNFR2, and iNOS contains binding sites for a number of transcription factors including NFκB (Carpentier et al., 2004; Kleinert et al., 2004; Makhatadze, 1998). We have recently demonstrated that CTS regulates the gene expression of proinflammatory molecules by affecting the NFκB pathway (Agarwal et al., 2003; Agarwal et al., 2004). Differences in the contribution of NFκB for the promoter activation of these genes or participation of other signaling molecules which are differentially affected by CTS might explain the observed diversity in gene expression of TNF-α, TNFR2, and iNOS in response to CTS under inflammatory conditions.

It is important to note that mechanical signals blocked the expression of TNF-α, TNFR2, and iNOS only transiently, i.e. these genes reexpressed following a defined period of rest in an inflammatory environment. For example, when cells were exposed to IL-1β for up to 48 h but subjected to CTS only for the initial 8 h, the expression of TNF-α and TNFR2 was inhibited at 24 h but not at 36 or 48 h. From these findings it might be speculated that mechanical loading even if needed only for a short time should be applied frequently to sustain its antiinflammatory effects.

Recently, we have found that the IL-1β-induced iNOS expression in articular chondrocytes was efficiently inhibited by CTS at low magnitudes, whereas application of CTS at higher magnitudes was required for similar inhibitory effects in knee meniscal cells (Ferretti et al., 2006; Madhavan et al., 2006). Our data suggest that TDC and meniscal cells are potentially less mechanosensitive compared to articular chondrocytes. TMJ disc and knee meniscus are composed of fibrocartilage, which markedly differs from hyaline cartilage in its cellular and biochemical composition, as well as biomechanical characteristics (Almarza and Athanasiou, 2004). The structural differences are strongly related to the distinct functions which these tissues have to serve and may explain the different response of fibrocartilaginous cells to biomechanical forces, as compared to articular chondrocytes. Since TMJ discs are difficult to visualize dynamically, data for in vivo deformations are lacking. However, disc elongations of 16±1% during mastication have been reported in minipigs (Sindelar and Herring, 2005). Similarly, strain of up to 25% have been measured in the minipig TMJ capsule during mastication (Liu and Herring, 2000).

In summary, our study provides first evidence that biomechanical signals block proinflammatory responses in TDC by suppressing the expression of TNFR2 but not TNFR1, and these responses are maintained for prolonged periods of time but are transient. Anti-arthritic treatment modalities targeting TNF-α actions such as anti-TNF antibodies and soluble TNFRs are currently applied to inhibit joint inflammation as well as prevent destruction of cartilage and bone. However, these are often only partially effective and have side effects which limit their usefulness (Keystone and Kavanaugh, 2005; O'Dell, 2004; Scheinfeld, 2005). Exercise or mechanical loading of joints are a promising approach to suppress pain and catabolic processes in joints (Israel and Syrop, 1997; Johnson and Eastwood, 1992; Kim et al., 1995). However, the critical questions regarding the duration of their effectiveness, the mechanisms of their actions, and target molecules that are affected

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have not been answered yet. Our observations may serve as a foundation for in vivo studies and demonstrate the necessity of using adequate time intervals for sustained effects of motion-based therapies in the optimal management of acute and chronic inflammation of joints.

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References

Abramson SB, Attur M, Amin AR, Clancy R. Nitric oxide and inflammatory mediators in the perpetuation of osteoarthritis. Current Rheumatological Report. 2001; 3:535–541.

Agarwal S, Deschner J, Long P, Verma A, Hofman C, Evans CH, Piesco N. Role of NF-kappaB transcription factors in anti-inflammatory and proinflammatory actions of mechanical signals. Arthritis and Rheumatism. 2004; 50:3541–3548. [PubMed: 15529376]

Agarwal S, Long P, Gassner R, Piesco NP, Buckley MJ. Cyclic tensile strain suppresses catabolic effects of interleukin-1beta in fibrochondrocytes from the temporomandibular joint. Arthritis and Rheumatism. 2001; 44:608–617. [PubMed: 11263775]

Agarwal S, Long P, Seyedain A, Piesco N, Shree A, Gassner R. A central role for the nuclear factor-kappaB pathway in anti-inflammatory and proinflammatory actions of mechanical strain. FASEB Journal. 2003; 17:899–901. [PubMed: 12670873]

Almarza AJ, Athanasiou KA. Design characteristics for the tissue engineering of cartilaginous tissues. Annals of Biomedical Engineering. 2004; 32:2–17. [PubMed: 14964717]

Atsu SS, Ayhan-Ardic F. Temporomandibular disorders seen in rheumatology practices: a review. Rheumatol International. 2006 [Epub ahead of print].

Campbell IK, Roberts LJ, Wicks IP. Molecular targets in immune-mediated diseases: the case of tumour necrosis factor and rheumatoid arthritis. Immunological Cell Biology. 2003; 81:354–366.

Carpentier I, Coornaert B, Beyaert R. Function and regulation of tumor necrosis factor type 2. Current Medicinal Chemistry. 2004; 11:2205–2212. [PubMed: 15279559]

Chowdhury TT, Bader DL, Lee DA. Dynamic compression counteracts IL-1 beta-induced release of nitric oxide and PGE2 by superficial zone chondrocytes cultured in agarose constructs. Osteoarthritis Cartilage. 2003; 11:688–696. [PubMed: 12954240]

Chowdhury TT, Bader DL, Lee DA. Anti-inflammatory effects of IL-4 and dynamic compression in IL-1beta stimulated chondrocytes. Biochemical and Biophysical Research Communication. 2006; 339:241–247.

Delgado-Molina E, Berini-Aytes L, Gay-Escoda C. Rheumatoid arthritis and temporomandibular joint involvement: a literature review. Medical Oral. 1997; 2:156–163.

Deschner J, Wypasek E, Ferretti M, Rath B, Anghelina M, Agarwal S. Regulation of RANKL by biomechanical loading in fibrochondrocytes of meniscus. Journal of Biomechanics. 2005 [Epub ahead of print].

Detamore MS, Athanasiou KA. Motivation, characterization, and strategy for tissue engineering the temporomandibular joint disc. Tissue Engineering. 2003a; 9:1065–1087. [PubMed: 14670096]

Detamore MS, Athanasiou KA. Structure and function of the temporomandibular joint disc: implications for tissue engineering. Journal of Oral and Maxillofacial Surgery. 2003b; 61:494–506. [PubMed: 12684970]

Fermor B, Jeffcoat D, Hennerbichler A, Pisetsky DS, Weinberg JB, Guilak F. The effects of cyclic mechanical strain and tumor necrosis factor alpha on the response of cells of the meniscus. Osteoarthritis Cartilage. 2004; 12:956–962. [PubMed: 15564062]

Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. Biorheology. 2002; 39:237–246. [PubMed: 12082286]

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Ferretti M, Madhavan S, Deschner J, Rath-Deschner B, Wypasek E, Agarwal S. Dynamic biophysical strain modulates proinflammatory gene induction in meniscal fibrochondrocytes. American Journal of Physiology and Cell Physiology. 2006; 290:C1610–C1615. [PubMed: 16452158]

Fu K, Ma X, Zhang Z, Chen W. Tumor necrosis factor in synovial fluid of patients with temporomandibular disorders. Journal of Oral and Maxillofacial Surgery. 1995; 53:424–426. [PubMed: 7699497]

Gassner R, Buckley M, Georgescu H, Studer R, Stefanovich-Racic M, Piesco N, Evans CH, Agarwal S. Cyclic tensile stress exerts antiinflammatory actions on chondrocytes by inhibiting inducible nitric oxide synthase. Journal of Immunology. 1999; 163:2187–2192.

Gassner RJ, Buckley MJ, Studer RK, Evans CH, Agarwal S. Interaction of strain and interleukin-1 in articular cartilage: effects on proteoglycan synthesis in chondrocytes. International Journal of Oral and Maxillofacial Surgery. 2000; 29:389–394. [PubMed: 11071247]

Giulietti A, Overbergh L, Valckx D, Decallonne B, Bouillon R, Mathieu C. An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. Methods. 2001; 25:386–401. [PubMed: 11846608]

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Analytical Biochemistry. 1982; 126:131–138. [PubMed: 7181105]

Grell M, Douni E, Wajant H, Lohden M, Clauss M, Maxeiner B, Georgopoulos S, Lesslauer W, Kollias G, Pfizenmaier K, Scheurich P. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. Cell. 1995; 83:793–802. [PubMed: 8521496]

Haas E, Grell M, Wajant H, Scheurich P. Continuous autotropic signaling by membrane-expressed tumor necrosis factor. Journal of Biological Chemistry. 1999; 274:18107–18112. [PubMed: 10364265]

Holtmann MH, Neurath MF. Differential TNF-signaling in chronic inflammatory disorders. Current Molecular Medicine. 2004; 4:439–444. [PubMed: 15354874]

Israel HA, Syrop SB. The important role of motion in the rehabilitation of patients with mandibular hypomobility: a review of the literature. Cranio. 1997; 15:74–83. [PubMed: 9586491]

Johnson DP, Eastwood DM. Beneficial effects of continuous passive motion after total condylar knee arthroplasty. Annals of the Royal College of Surgeons of England. 1992; 74:412–416. [PubMed: 1471839]

Kay J, Calabrese L. The role of interleukin-1 in the pathogenesis of rheumatoid arthritis. Rheumatology. 2004; 43(Suppl 3):iii2–iii9. [PubMed: 15150426]

Keystone EC, Kavanaugh A. What to do with TNF failures. Expert Opinion in Drug Safety. 2005; 4:149–155.

Kim HK, Kerr RG, Cruz TF, Salter RB. Effects of continuous passive motion and immobilization on synovitis and cartilage degradation in antigen induced arthritis. Journal of Rheumatol. 1995; 22:1714–1721.

Kleinert H, Pautz A, Linker K, Schwarz PM. Regulation of the expression of inducible nitric oxide synthase. European Journal of Pharmacology. 2004; 500:255–266. [PubMed: 15464038]

LeGrand A, Fermor B, Fink C, Pisetsky DS, Weinberg JB, Vail TP, Guilak F. Interleukin-1, tumor necrosis factor alpha, and interleukin-17 synergistically up-regulate nitric oxide and prostaglandin E2 production in explants of human osteoarthritic knee menisci. Arthritis and Rheumatism. 2001; 44:2078–2083. [PubMed: 11592370]

Liu ZJ, Herring SW. Masticatory strains on osseous and ligamentous components of the temporomandibular joint in miniature pigs. Journal of Orofacial Pain. 2000; 14:265–278. [PubMed: 11203760]

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods. 2001; 25:402–408. [PubMed: 11846609]

Long P, Gassner R, Agarwal S. Tumor necrosis factor alpha-dependent proinflammatory gene induction is inhibited by cyclic tensile strain in articular chondrocytes *in vitro*. Arthritis and Rheumatism. 2001; 44:2311–2319. [PubMed: 11665971]

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Madhavan S, Anghelina M, Rath-Deschner B, Wypasek E, John A, Deschner J, Piesco NP, Agarwal S. Biomechanical signals exert sustained attenuation of proinflammatory gene induction in articular chondrocytes. Osteoarthritis Cartilage. 2006 Epub ahead of print.

Makhatadze NJ. Tumor necrosis factor locus: genetic organisation and biological implications. Human Immunology. 1998; 59:571–579. [PubMed: 9757913]

McDevitt C, Miller R. Biochemistry, cell biology, and immunology of osteoarthritis. Current Opinion in Rheumatology. 1989; 1:303–314. [PubMed: 2701683]

Miyasaka N, Hirata Y. Nitric oxide and inflammatory arthritides. Life Sciences. 1997; 61:2073–2081. [PubMed: 9395248]

O'Dell JR. Therapeutic strategies for rheumatoid arthritis. New England Journal of Medicine. 2004; 350:2591–2602. [PubMed: 15201416]

Salter RB. History of rest and motion and the scientific basis for early continuous passive motion. Hand Clinics. 1996; 12:1–11. [PubMed: 8655611]

Scheinfeld N. Adalimumab: a review of side effects. Expert Opinion in Drug Safety. 2005; 4:637–641.

Sindelar BJ, Herring SW. Soft tissue mechanics of the temporomandibular joint. Cells Tissues Organs. 2005; 180:36–43. [PubMed: 16088132]

Takahashi K, Kubo T, Arai Y, Kitajima I, Takigawa M, Imanishi J, Hirasawa Y. Hydrostatic pressure induces expression of interleukin 6 and tumour necrosis factor alpha mRNAs in a chondrocyte-like cell line. Annals of the Rheumatic Diseases. 1998; 57:231–236. [PubMed: 9709180]

Tanaka E, van Eijden T. Biomechanical behavior of the temporomandibular joint disc. Critical Reviews in Oral Biology and Medicine. 2003; 14:138–150. [PubMed: 12764076]

Westacott C, Urban J, Goldring M, Elson C. The effects of pressure on chondrocyte tumour necrosis factor receptor expression. Biorheology. 2002; 39:125–132. [PubMed: 12082275]

Xu Z, Buckley M, Evans C, Agarwal S. Cyclic tensile strain acts as an antagonist of IL-1 beta actions in chondrocytes. Journal of Immunology. 2000; 165:453–460.

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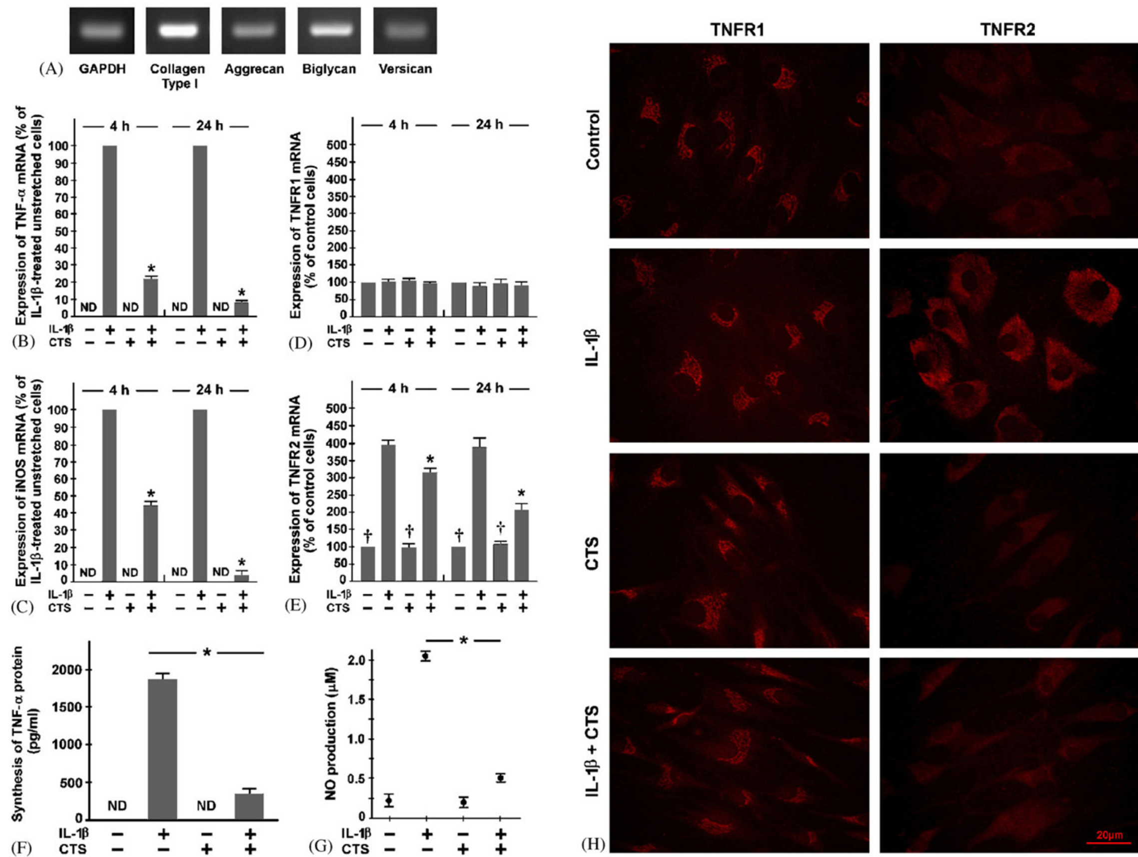

Fig. 1.

(A) Expression of mRNA for GAPDH, collagen type I, aggrecan, biglycan, and versican in untreated TDC, as analyzed by end-point RT–PCR. Expression of mRNA for (B) TNF-α, (C) iNOS, (D) TNFR1, and (E) TNFR2 in TDC exposed to IL-1β (1 ng/ml) and/or CTS (20%, 0.05 Hz) at 4 and 24 h, as analyzed by real-time RT–PCR. Untreated cells served as controls. Results are shown as means±SEM, $n = 6$, ND: below the detection limit of real-time RT–PCR, * significantly different from IL-1β-treated unstretched cells (p<0.05), † significantly different from IL-1β-treated unstretched cells and IL-1β-treated cells simultaneously exposed to CTS (p<0.05). (F) Concentration of TNF-α protein in the culture supernatants of cells subjected to treatment with IL-1β and/or CTS at 24 h, as measured by ELISA. Untreated cells served as controls. Results are shown as means±SEM, $n = 6$, ND: below the detection limit of real-time RT–PCR, * significant difference between IL-1β-treated cells exposed to CTS and IL-1β-treated unstretched cells (p<0.05). (G) NO levels in culture supernatants of cells subjected to treatment with IL-1β and/or CTS at 24 h, as determined by Griess reaction. Untreated cells served as controls. Results are shown as means±SEM, $n = 6$, * significant difference between IL-1β-treated cells exposed to CTS and IL-1β-treated unstretched cells (p<0.05). (H) Protein synthesis of TNFR1 and TNFR2 in cells exposed to IL-1β and/or CTS at 24 h, as assessed by immunofluorescence. Untreated cells served as controls. (A–H) Data from one of three representative experiments are presented.

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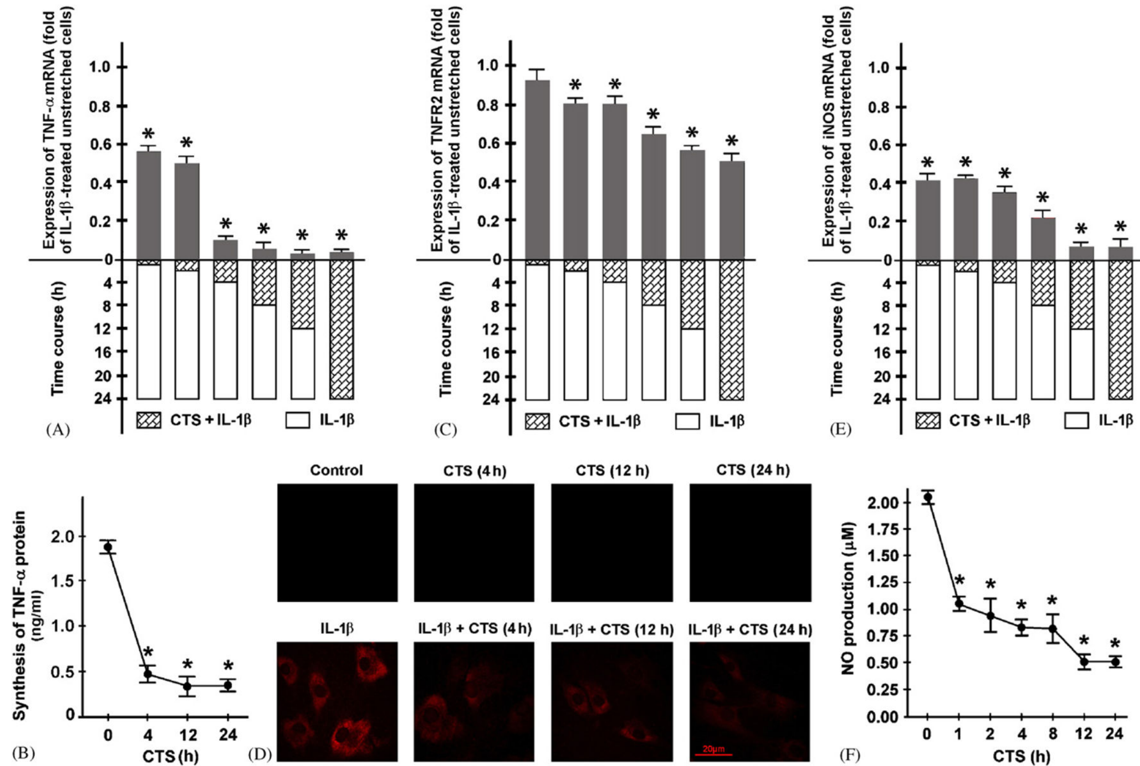

Fig. 2.

Expression of mRNA for (A) TNF-α, (C) TNFR2, and (E) iNOS at 24 h, as analyzed by real-time RT–PCR. Cells were treated with IL-1β for 24 h while being subjected to CTS either only for the initial 1, 2, 4, 8, and 12 h or for the entire 24 h-interval. IL-1β-treated unstretched cells served as controls. Results are shown as means±SEM, $n = 6$, * significant difference between IL-1β-treated cells exposed to CTS and IL-1β-treated unstretched cells. (B) Concentration of TNF-α protein in the culture supernatants of cells at 24 h, as measured by ELISA. Cells were treated with IL-1β for 24 h while being subjected to CTS either only for the initial 4 and 12 h or the entire 24 h-interval. IL-1β-treated unstretched cells served as controls. Results are shown as means±SEM, $n = 6$, * significant difference between IL-1β-treated cells exposed to CTS and IL-1β-treated unstretched cells. (D) Protein synthesis of TNFR2 at 24 h, as assessed by immunofluorescence. Cells were treated with IL-1β for 24 h while being subjected to CTS either only for the initial 4 and 12 h or the entire 24 h-interval. IL-1β-treated unstretched cells, cells exposed to CTS in the absence of IL-1β, and untreated cells served as controls. (F) NO levels in culture supernatants at 24 h, as measured by Griess reaction. Cells were treated with IL-1β for 24 h while being exposed to CTS either only for the initial 1, 2, 4, 8, and 12 h or the entire 24 h-interval. IL-1β-treated unstretched cells served as controls. Results are shown as means±SEM, $n = 6$, * significant difference between IL-1β-treated cells exposed to CTS and IL-1β-treated unstretched cells. (A–F) Data from one of three representative experiments are presented.

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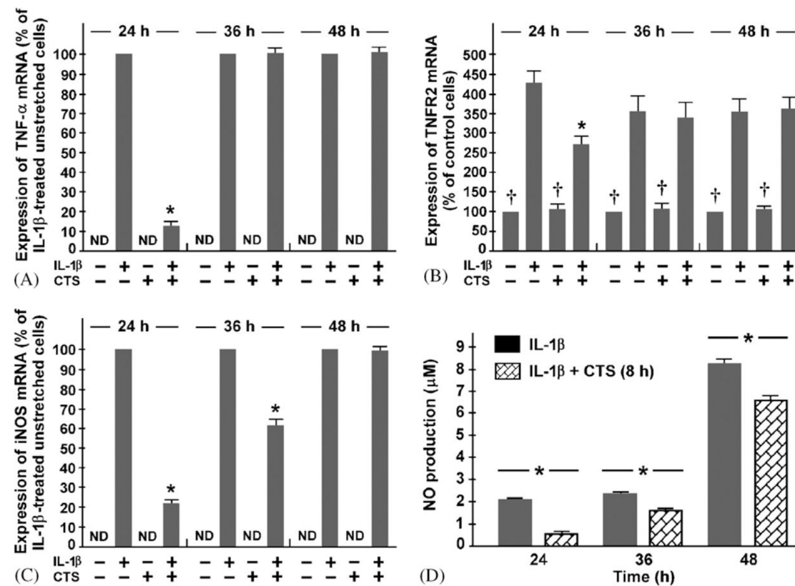

Fig. 3.

Expression of mRNA for (A) TNF-α, (B) TNFR2, and (C) iNOS at 24, 36, and 48 h, as analyzed by real-time RT–PCR. Cells were incubated with IL-1β for 24, 36, and 48 h, respectively, while CTS was applied only for the initial 8 h of the entire incubation time. IL-1β-treated unstretched cells, cells exposed to CTS in the absence of IL-1β, and untreated cells served as controls. Results are shown as means±SEM, $n = 6$, ND: below the detection limit of real-time RT–PCR, * significantly different from IL-1β-treated unstretched cells ($p<0.05$). † significantly different from IL-1β-treated unstretched cells and IL-1β-treated cells simultaneously exposed to CTS ($p<0.05$). (D) NO levels in culture supernatants at 24, 36, and 48 h, as measured by Griess reaction. Cells were treated with IL-1β for 24, 36, and 48 h, respectively, while being exposed to CTS only for the initial 8 h of the entire IL-1β incubation time. IL-1β-treated unstretched cells served as controls. Results are shown as means±SEM, $n = 6$, * significant difference between IL-1β-treated cells exposed to CTS and IL-1β-treated unstretched cells. (A–D) Data from one of three representative experiments are presented.

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Table 1

Primer sequences for end-point RT–PCR

Molecule	Primer sequences		Amplicon length	Accession no.
GAPDH	Sense (5′-3′) Antisense (5′-3′)	AGA CAG CCG CAT CTT CTT GT TAC TCA GCA CCA GCA TCA CC	323	X02231
Collagen Type I	Sense (5′-3′) Antisense (5′-3′)	CTG CTG GAG AAC CTG GAA AG GGA AAC CTC TCT CGC CTC TT	282	Z78279
Aggrecan	Sense (5′-3′) Antisense (5′-3′)	CTA CGA CGC CAT CTG CTA CA GCT TTG CAG TGA GGA TCA CA	179	NM_022190
Biglycan	Sense (5′-3′) Antisense (5′-3′)	CAG GCT CAG ACA CCA CTT CA ACT TTG CGG ATA CGG TTG TC	400	U17834
Versican	Sense (5′-3′) Antisense (5′-3′)	GTG GAA GGC ACA GCA GTT TA TGT AGC CAG GTG CAC AGG T	121	U75306

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Table 2

Primer sequences for real-time RT–PCR

Molecule	Primer and probe sequences		Amplicon length	Accession no.
TNF-α	Sense (5′–3′)	CCC AGA CCC TCA CAC TCA GAT C	85	X66539
	Antisense (5′–3′)	TCG TAG CAA ACC ACC AAG CAG		
	Probe (5′–3′)	6-FAM d(TCGAGTGACAAGCCCGTAGCCCA)BHQ-1		
TNFR1	Sense (5′–3′)	TCA CCG GAC TGG TTC CTT CT	85	NM_013091
	Antisense (5′–3′)	TCT TTG GAT GGG CAT ACT TTC C		
	Probe (5′–3′)	6-FAM d(TTGGTGACCGGGAGAAGAGGGATAATTTG)BHQ-1		
TNFR2	Sense (5′–3′)	CCT GGA GCA CTC TGT CTA ATT CC	85	AF498039
	Antisense (5′–3′)	ACA GTG CAT AAC CCG GAC ATG		
	Probe (5′–3′)	6-FAM d(CCACTTGGTACTGTACTCGCTGTGA)BHQ-1		
iNOS	Sense (5′–3′)	TTC TGT GCT AAT GCG GAA GGT	85	D44591
	Antisense (5′–3′)	GCT TCC GAC TTT CCT GTC TCA		
	Probe (5′–3′)	6-FAM d(CCGCGTCAGAGCCACAGTCCT)BHQ-1		
GAPDH	Sense (5′–3′)	CTC AAC TAC ATG GTC TAC ATG TTC CA	85	X02231
	Antisense (5′–3′)	CTT CCC ATT CTC AGC CTT GAC T		
	Probe (5′–3′)	HEX d(ACCCACGGCAAGTTCAACGGCA)BHQ-1		