Effects of TGF-β1 and IL-1β on expression of ADAMTS enzymes and TIMP-3 in human intervertebral disc degeneration

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Received June 25, 2013; Accepted September 27, 2013

DOI: 10.3892/etm.2013.1348

Abstract. The aim of this study was to investigate the effects of transforming growth factor-β1 (TGF-β1) and interleukin-1β (IL-1β) on the expression of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) enzymes and their inhibitor, tissue inhibitor of metalloproteinase 3 (TIMP-3), in human intervertebral disc (IVD) degeneration. Cells from patients with IVD degeneration were cultured with Dulbecco's modified Eagle's medium with Ham's F12 nutrient mixture (DMEM/F12) medium at 37˚C in a 5% CO2 incubator. Cell proliferation was measured by cell counting kit-8 assays with varying concentrations of TGF-β1 and IL-1β in a time-response experiment. The mRNA and protein expression levels of ADAMTS-4, ADAMTS-5 and TIMP-3 were detected with qPCR and western blot analysis, respectively. The present study demonstrated that TGF-β1 promoted nucleus pulposus (NP) cell proliferation, decreased the expression of ADAMTS-4 and -5 and increased the expression of TIMP-3. By contrast, the IL-1β treatment inhibited NP cell proliferation and significantly increased the expression of ADAMTS-4 and -5. However, IL-1β appeared to have no marked effect on the expression of TIMP-3. This study suggests that TGF-β1 and IL-1β are involved in the synthesis and degradation of the extracellular matrix and may act as potential therapeutic targets for the prevention or reversal of IVD degeneration.

Introduction

The human intervertebral disc (IVD) is an important component of the spinal column and its dysfunction leads to lower back pain that may reduce the patient's quality of life (1). The degeneration of the IVD is a complex process characterized by a series of biochemical and structural changes in the nucleus pulposus (NP) (2). IVD degeneration often leads to the unclear boundary between the annulus fibrous and NP, composition changes of the collagen fibers, as well as a reduction in the proteoglycan content and loss of water (3). NP cells are chondrocyte-like cells and secrete a complex extracellular matrix (ECM) that predominantly consists of proteoglycan and fibrillar collagen. Aggrecan, one type of proteoglycan, maintains the normal structure, metabolism and biomechanical function of the disc (4). Glicosaminoglycan (GAG), a component of proteoglycans, contains large quantities of water, which allows the NP to be flexible enough to withstand loading. The considerable loss of GAG in the process of disc degeneration results in reductions in water content and NP elasticity, which lead to the dysfunction of IVD biomechanics (5). A previous study revealed that the loss of GAG predominantly results from the increased hydrolysis of proteoglycans (6). Aggrecan synthesis and degradation are in a dynamic equilibrium that maintains the physiological function of the IVD. However, this dynamic balance gradually becomes distorted under the influence of age and stress, resulting in the degeneration of human IVDS. A previous study has demonstrated that the expression and activity of matrix-degrading enzymes are increased and elicit degradation of the ECM in the degenerative disc (7).

There are two predominant degrading enzymes that are able to hydrolyze aggrecan core proteins; these are matrix metalloproteinases (MMPs) and aggrecanases (8). MMPs have been demonstrated to hydrolyze aggrecan and collagen, as well as fibroectin proteins. Aggrecanase belongs to the a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family and has the ability to degrade aggrecan. In the NP matrix, aggrecan is predominantly degraded by the ADAMTS family members ADAMTS-4 and ADAMTS-5 (8,9). Tissue inhibitor of metalloproteinase 3 (TIMP-3), inhibits aggrecanase and therefore, an increase in the level of TIMP-3 slows disc degeneration. Studies have demonstrated that growth factors, including transforming growth factor-β1 (TGF-β1), and cytokines, such as interleukin-1β (IL-1β), are involved in ECM metabolism and cell proliferation (10,11). The present study investigated the effects of TGF-β1 and IL-1β on the expression levels of ADAMTS enzymes and their inhibitor TIMP-3 in human IVD degeneration, and aimed to identify a potential therapeutic target for human IVD degeneration.
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Statistical analysis. Data are presented as the mean ± SEM and were analyzed using SPSS software (SPSS, Inc., Chicago, IL, USA). Student's t-test and analysis of variance were used for comparisons between groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Effects of TGF-β1 and IL-1β on the proliferation of human NP cells. To determine the effect of TGF-β1 and IL-1β on NP cell proliferation, a time-response experiment of human NP cell proliferation was performed using the CCK-8 assay in 96-well plates. Fig. 1 demonstrates that treatment with TGF-β1 at a concentration of 0.1 ng/ml exhibited no significant effect on the proliferation of NP cells. However, a high concentration of TGF-β1 (1 and 10 ng/ml) treatment significantly promoted cell proliferation. Furthermore, Fig. 2 shows that 10 ng/ml IL-1β significantly inhibited the human NP cell proliferation, while lower concentrations of IL-1β did not exhibit a marked effect. These results revealed that 1 or 10 ng/ml for TGF-β1 and 10 ng/ml for IL-1β appeared to be the optimal concentrations for the inhibition of human NP cell proliferation and were applied in the subsequent experiment.

Effects of TGF-β1 on the expression of ADAMTS enzymes and TIMP-3 in human NP cells. Human IVD degeneration is accompanied by the increased expression of degrading enzymes, including ADAMTS. Therefore, the effect of TGF-β1 on the expression of ADAMTS-4 and ADAMTS-5, the enzymes that are predominantly involved in the matrix degrading process, was examined. qPCR showed that the expression of ADAMTS-4 and -5 mRNA significantly decreased in a time-dependent manner following treatment with TGF-β1 at a concentration of 1 ng/ml. Western blot analysis also demonstrated that the protein expression of ADAMTS-4 and -5 significantly decreased following treatment with TGF-β1 for 48 h (Fig. 3A and B). However, treatment with 10 ng/ml TGF-β1 appeared to increase the expression of the ADAMTS enzymes (Fig. 3C and D), indicating that a high concentration of TGF-β1 may produce an adverse effect. Subsequently, the expression of the MMP inhibitor, TIMP-3, was examined and the results revealed that TGF-β1 treatment at concentrations of 1 and 10 ng/ml (data not shown for the latter) significantly increased the expression of TIMP-3 at the mRNA level in a time-dependent manner. The western blot analysis revealed that the TIMP-3 protein levels were increased following treatment with 1 ng/ml TGF-β1 for 48 h (Fig. 3E).

Effects of IL-1β on the expression of ADAMTS enzymes and TIMP-3 in human NP cells. The effects of IL-1β on the expression of ADAMTS-4, ADAMTS-5 and TIMP-3 in the NP cells were examined. qPCR revealed that the expression of ADAMTS-4 and -5 significantly increased in a time-dependent manner following treatment with IL-1β at a concentration of 10 ng/ml. Western blot analysis also demonstrated that the protein expression levels of ADAMTS-4 and -5 significantly increased with 48 h of IL-1β treatment (Fig. 4A and B). However, IL-1β appeared to have no marked effect on the expression of TIMP-3 (Fig. 4C).

Discussion

IVD degeneration is characterized by morphological changes and is closely associated with the degradation of the ECM, which leads to a reduction in the NP cell population (13). The proteoglycan (predominantly aggrecan) content is important in maintaining proper IVD function, particularly in the NP (5). The role of MMPs in the articular cartilage and IVD ECM has been studied for a number of years and is relatively well understood. Several members of the aggrecanase enzyme family that participate in ECM breakdown have been identified, and studies have shown that aggrecanases may be involved in the initiation and progression of IVD degeneration (14). ADAMTS-4 and ADAMTS-5 are critical in the breakdown of ECM in the degenerative disc (15,16). The expression of TIMP-3 has been identified to be reduced in degenerated NP and annulus fibrosus samples and is known to inhibit the two ADAMTS enzymes and numerous other matrix proteinases, such as MMPs (17).

Cytokines are critical in ECM synthesis as they regulate cell metabolism. TGF-β1 has been shown to stimulate NP cell proliferation and has been applied in tissue engineering for the repair of IVD. TGF-β1 treatment has been demonstrated to decrease mitogen-activated protein kinase activity and sex determining region Y-box 9, aggrecan, and collagen type II...
gene expression (18). In addition, another study reported that in IVD cells, stimulation by TGF-β1, epidermal growth factor and insulin-like growth factor may significantly increase proteoglycan synthesis and promote cell proliferation (17,19). IL-1β plays a biological role by promoting cell metabolism and inhibiting anabolism. It stimulates cells to produce a variety of neutral proteases, particularly MMPs, and reduce the expression of proteoglycan in the ECM (11,13,20). Studies have shown that IL-1β is expressed in degenerating discs and is involved in the catabolism of the ECM. Tsuji et al (21) showed that the stimulation of NP cells with IL-1β for 24 h significantly increased the expression level of ADAMTS-4 mRNA and demonstrated that IL-1β is involved in the degradation of ECM in the IVD cells. Therefore, it was hypothesized that the inhibition of IL-1β expression may be a treatment option for IVD degeneration. The present study aimed to investigate the...
involvement of TGF-β1 and IL-1β in the proliferation of NP cells and expression of genes involved in ECM synthesis. The results demonstrated that TGF-β1 promoted NP cell proliferation. Furthermore, the downregulation of ADAMTS-4 and -5 and upregulation of TIMP-3 was demonstrated following treatment with 1 ng/ml TGF-β1. However, treatment with a higher concentration of TGF-β1 (10 ng/ml) appeared to increase the expression of ADAMTS enzymes, indicating that an over-dose of TGF-β1 treatment may produce an adverse effect. Conversely, IL-1β treatment inhibited NP cell proliferation and significantly increased the expression of ADAMTS-4 and -5. However, IL-1β appeared to have no marked effect on the expression of TIMP-3.

In conclusion, this study demonstrated that the cytokines TGF-β1 and IL-1β, are involved in the synthesis and degradation of the ECM and may be potential therapeutic targets for the prevention or reversal of IVD degeneration.

References

1. Speed C: Low back pain. BMJ 328: 1119-1121, 2004.
2. Tolonen J, Grönblad M, Vanharanta H, Virri J, Geyer RD, Rytömaa T and Karaharju EO: Growth factor expression in degenerated intervertebral disc tissue. An immunohistochemical analysis of transforming growth factor beta, fibroblast growth factor and platelet-derived growth factor. Eur Spine J 15: 588-596, 2006.
3. Fraser RD, Osti OL and Vernon-Roberts B: Intervertebral disc degeneration. Eur Spine J 1: 205-213, 1993.
4. Le Maître CL, Pockert A, Buttle DJ, Freemont AJ and Hoyland JA: Matrix synthesis and degradation in human intervertebral disc degeneration. Biochem Soc Trans 35: 652-655, 2007.
5. Roughley PJ, Alini M and Antoniou J: The role of proteoglycans in aging, degeneration and repair of the intervertebral disc. Biochem Soc Trans 30: 869-874, 2002.
6. Inkinen RI, Lammi MJ, Lehmonen S, Puustjärvi K, Kääpä E and Tammi MI: Relative increase of biglycan and decorin and altered chondroitin sulfate epitopes in the degenerating human intervertebral disc. J Rheumatol 25: 506-514, 1998.
7. Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC and Eisenstein SM: Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc. Spine (Phila Pa 1976) 25: 3005-3013, 2000.
8. Patel KP, Sandy JD, Akeda K, Miyamoto K, Chuju T, An HS and Masuda K: Aggrecanases and aggrecanase-generated fragments in the human intervertebral disc at early and advanced stages of disc degeneration. Spine (Phila Pa 1976) 32: 2596-2603, 2007.
9. Hatano E, Fujita T, Ueda Y, Okuda T, Katsuda S, Okada Y and Matsumoto T: Expression of ADAMTS-4 (aggrecanase-1) and possible involvement in regression of lumbar disc herniation. Spine (Phila Pa 1976) 31: 1426-1432, 2006.
10. Chen WH, Lo WC, Lee JJ, Su CH, Lin CT, Liu HY, Lin TW, Lin WC, Huang TY and Deng WP: Tissue-engineered intervertebral disc and chondrogenesis using human nucleus pulposus regulated through TGF-beta1 in platelet-rich plasma. J Cell Physiol 209: 744-754, 2006.
11. Le Maître CL, Freemont AJ and Hoyland JA: The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration. Arthritis Res Ther 7: R73-R74, 2005.
12. Christian WA, Alexander M, Marco Z: Magnetic resonance classification of 275 lumbar intervertebral disc degeneration. Spine (Phila Pa 1976) 17: 1873-1878, 276 2001.
13. Kluba T, Niemeyer T, Gaissermaier C and Gründer T: Human anulus fibrosis and nucleus pulposus cells of the intervertebral disc: effect of degeneration and culture system on cell phenotype. Spine (Phila Pa 1976) 30: 2743-2748, 2005.
14. Pockert AJ, Richardson SM, Le Maître CL, Lyon M, Deakin JA, Buttle DJ, Freemont AJ and Hoyland JA: Modified expression of the ADAMTS enzymes and tissue inhibitor of metalloproteinases 3 during human intervertebral disc degeneration. Arthritis Res 60: 482-491, 2009.
15. Majumdar MK, Askew R, Schelling S, Stedman N, Blanchet T, Hopkins B, Morris EA and Glasson SS: Double-knockout of ADAMTS-4 and ADAMTS-5 in mice results in physiologically normal animals and prevents the progression of osteoarthritis. Arthritis Rheum 56: 3670-3674, 2007.
16. Stanton H, Rogerson FM, East CJ, Golub SB, Lawlor KE, Meeker CT, Little CB, Last K, Farmer PJ, Campbell IK, et al: ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. Nature 434: 648-652, 2005.
17. Osada R, Ohshima H, Ishihara H, Yudoh K, Sakai K, Matsu H and Tsuji H: Autocrine/paracrine mechanism of insulin-like growth factor-1 secretion, and the effect of insulin-like growth factor-1 on proteoglycan synthesis in bovine intervertebral discs. J Orthop Res 14: 690-699, 1996.
18. Rishbud MV, Albert TJ, Guttapalli A, Vresilovic EJ, Hillibrand AS, Vaccaro AR and Shapiro IM: Differentiation of mesenchymal stem cells towards a nucleus pulposus-like phenotype in vitro: implications for cell-based transplantation therapy. Spine (Phila Pa 1976) 29: 2627-2632, 2004.
19. Gruber HE, Fischer EC Jr, Desai B, Stasky AA, Hoelscher G and Hanley EN Jr: Human intervertebral disc cells from the annulus: three-dimensional culture in agarose or alginate and responsiveness to TGF-beta1. Exp Cell Res 235: 13-21, 1997.
20. Burke JG, G Watson RW, Conhyea D, McCormack D, Burke JG, G Watson RW, Conhyea D, McCormack D and Hanley EN Jr: Human intervertebral disc cells from the annulus: three-dimensional culture in agarose or alginate and responsiveness to TGF-beta1. Exp Cell Res 235: 13-21, 1997.
21. Tsuji T, Chiba K, Imabayashi H, Fujita Y, Hosogane N, Okada Y and Toyama Y: Age-related changes in expression of tissue inhibitor of metalloproteinases-3 associated with transition from the notochordal nucleus pulposus to the fibrocartilaginous nucleus pulposus in rabbit intervertebral disc. Spine (Phila Pa 1976) 32: 849-856, 2007.