INTRODUCTION

Traumatic injury to cartilage is often associated with acutely elevated levels of pro-inflammatory cytokines, such as interleukin-1 (IL-1), that promote the accumulation and activation of degradative enzymes, including collagenases (MMP-1, MMP-13) and aggrecanases (ADAMTS-4, ADAMTS-5) in the synovial joint [1]. These enzymes prompt degradation of major extracellular matrix components, which leads to the initiation and progression of osteoarthritis [2]. The activation of aggrecanases is effectively regulated by an endogenous inhibitor, called tissue inhibitor of metalloproteinases 3 (TIMP-3) [3]. Recent studies show that TIMP-3 can also bind to collagenases with undefined affinity [4]. However, the role of TIMP-3 in prevention of cartilage degradation remains unclear. In this study, we hypothesize that TIMP-3 would prevent proteoglycan loss and collagen degradation induced by IL-1 and post-traumatic injury. The specific aims of this study were to determine the efficacy of the ability of TIMP-3 to inhibit MMP-1 and MMP-13, and to determine the efficacy of TIMP-3 on preventing both IL-1 induced and post-injury (in the presence and absence of loading) cartilage degradation.

MATERIALS AND METHODS

The apparent collagenase activity of MMP-1 and MMP-13 (Chemicon) were assessed using a FRET substrate kit (Anaspec). The MMPs at the level of 0, 0.5, 1, 2, or 4 nM were activated and co-incubated with FRET substrates and varying concentrations of human recombinant TIMP-3 (0, 4, 8, 12, 16, 24, and 32 nM). The inhibition constant (K_i) and kinetic properties (K_{cat}, K_{m}, and K_{cat}/K_{m}) were calculated using Michaelis-Menten kinetic equations. To determine the efficacy of TIMP-3 on preventing IL-1 and injury-induced proteoglycan loss, bovine explants and an in vitro load system were used. Mature bovine (18-24 months old) knee joints were obtained from a local abattoir. Cartilage explants were harvested from the trochlear groove using a 7-mm biopsy punch. Explants were cultured in DMEM with ITS+ (serum-free) supplement for at least 24 hrs before treatments. To determine the efficacy of TIMP-3 on preventing IL-1 proteoglycan loss and collagenase activities, cartilage explants were treated with IL-1 (10 ng/mL) and varying levels of human recombinant TIMP-3 (0, 30, 300, 3000 nM) for 48 hrs. Culture medium was collected and analyzed for proteoglycan content and apparent collagen activity using FRET substrate kit (Anaspec). An additional 50 explants were used to assess the efficacy of TIMP-3 on preventing injury-induced cartilage degradation in the presence of load. These explants were divided into 10 groups, including: free swelling (FS), load (L), injury (I), injury+load (IL), and IL-1, all with and without treatment with 1000 nM of TIMP-3. Injury (30-40% cell death) was induced by 60% decompensation while 0.5 MPa confined compression was used to load cartilage as previously described [5]. Culture media was then assessed for proteoglycan loss, and explants were snap-frozen for mRNA expression (MMP-1, MMP-13, ADAMTS-4, and ADAMTS-5) or fixed for histological analysis (Safranin-O/Fast Green and collagen alignment).

RESULTS

Results from the kinetic study suggested that MMP-13 was significantly (>2 fold) more active than MMP-1 and TIMP-3 had a greater inhibitory efficiency on MMP-13 (K_i=0.58±0.08 nM) than MMP-1 (K_i=3.8±0.6 nM). From the dose-dependent study, we found that IL-1 induced proteoglycan loss was significantly inhibited with increasing levels of TIMP-3 (p<0.001, Figure 1A) and that over 90% apparent collagenase activity was inhibited by TIMP-3 with concentrations over 300 nM (Figure 1B). In the study of post-injury cartilage degradation, DMMB results showed that TIMP-3 reduced proteoglycan loss in injury and IL-1 treatment groups (Figure 2). Histological analysis with Safranin-O/Fast Green demonstrated that proteoglycan loss was primarily localized to the superficial zone in non-treated groups. Furthermore, collagen was shown to be misaligned and degraded in the non-treated groups, as indicated by the absence of a distinct superficial staining band, while groups exposed to TIMP-3 retained appropriate collagen alignment (Figure 3). In the analysis of mRNA expression, the treatment of TIMP-3 did not affect the expression of MMP-1, MMP-13 and ADAMTS-4 and ADAMTS-5. This suggests that the inhibition of collagenases and aggrecanases is due to binding to the enzyme with minimal effects on gene expression.

DISCUSSION

These findings support our hypothesis that the treatment of TIMP-3 prevents proteoglycan loss and collagen degradation induced by IL-1 and post-traumatic injury. More specifically, it appears that TIMP-3 can inhibit collagenases, including MMP-1 and MMP-13, in addition to aggrecanases. Although it has been shown that TIMP-3 has some ability to inhibit collagenase activities, our findings suggested that inhibition by TIMP-3 is more effective on MMP-13 than MMP-1. Together, we found that TIMP-3 can provide protection against aggressive degradation of proteoglycans and collagen due to elevation of IL-1 and traumatic insult after injury. This suggests that TIMP-3 is a potential treatment for preventing post-traumatic osteoarthritis and other types of arthritis.

REFERENCES


ACKNOWLEDGEMENTS

This study was supported in part by NIH (CTC-AR50549) and by a grant from the New York Chapter of the Arthritis Foundation.