TGF-β1 Decreases Plasmin-Mediated MMP-2 Activity in Flexor Tendon Cells: Implications for Scarless Tendon Repair

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Disclosures:

Introduction: Flexor tendon injuries caused by deep lacerations to the hands are a challenging problem for tissue engineers. Such injuries often result in debilitating adhesions, scar tissue that prevents the normal gliding motion of the flexor tendons through their lubricating synovial sheaths, preventing the hands from opening and closing. Evidence exists that tendon adhesions as well as scarring throughout the body are largely mediated by the pleiotropic growth factor, TGF-β1 [1], the effects of which are poorly understood in tendon. Using an in vitro model of tendon healing, we previously found that TGF-β1 causes gene expression changes in tenocytes that are consistent with scar tissue and adhesion formation [2], and upregulates the antifibrinolytic protein, PAI-1. PAI-1 is thought to contribute to tissue fibrosis by directly suppressing the activity of the fibrinolytic enzyme, plasmin, and additionally preventing the degradation of ECM proteins such as collagen and fibronectin by reducing plasmin-mediated MMP activity [3]. Therefore, we hypothesized that TGF-β1 contributes to scarring and adhesions in the flexor tendons by upregulating PAI-1 and thereby suppressing plasmin activity and plasmin-mediated MMP activity. To test this hypothesis, we examined the effects of TGF-β1 on plasminogen activation to plasmin, MMP-2 expression and activity, and the expression of tPA, uPA and PAI-1 in tenocytes cultured with combinations of TGF-β1, plasminogen, and tPA.

Methods: Tissue Harvest & Cell Culture: All animals were cared for in compliance with regulations of the University Committee on Animal Research. Flexor digitorum longus tendons were obtained from five freshly sacrificed, 7 month old C57BL/6 mice, and processed for tendon cell (i.e. tenocyte) culture as described previously [2]. Experimental Model: 6-well plates were pre-coated with 50 µg/cm2 of rat tail tendon collagen I (BD Biosciences, #354236). Tenocytes were seeded onto the plates (70,000 cells/well) and incubated overnight in control media (MEM α and 1% FBS). The next day, time 0 samples were collected, and the remaining samples were treated with fresh control media with or without 10 ng/mL of TGF-β1 (R&D Systems, #240-B-010), 20 µg/mL of human glu-plasminogen (Haematologic Technologies, HCPG-0130), and 50 ng/mL of tPA (Technoclone, #TC41072). Cell and media samples were collected and processed after 48 h to assess gene expression with RT-PCR, Total PAI-1 (Molecular Innovations) and glu-plasminogen (Technoclone, TC12040) protein levels with ELISA, plasmin activity with a fluorogenic substrate assay (AnaSpec, #72125), and MMP-2 activity with gelatin zymography (Invitrogen). Statistical Analysis: Gelatin zymograms were quantified in ImageJ, and band densities were analyzed, along with gene expression and protein levels, using 1-way ANOVA and Bonferroni post-tests in GraphPad Prism 4. Statistical significance was defined as p<0.05.

Results: PAI-1 Protein Levels: PAI-1 protein levels in culture media were significantly elevated by 13- to 33-fold in all groups treated with TGF-β1, including those supplemented with plasminogen and tPA (Figure 1A). Plasmin Activity: TGF-β1 reduced active plasmin levels by as much as 82% in groups that were supplemented with exogenous Plasminogen and tPA (Figure 1A). Plasminogen Protein Levels: tPA caused an 85% decrease in plasminogen compared to non-tPA treated controls. Upon addition of TGF-β1, tPA only caused a 45% decrease in plasminogen compared to non-tPA treated controls. MMP Activity: Active and Pro-MMP-2 in culture media samples were measured with Gelatin Zymography (Figure 1C). TGF-β1 reduced active MMP-2 by ~83% in the presence of Plasminogen with or without tPA, however, significant differences were only achieved among groups treated with tPA. Gene Expression: TGF-β1 significantly upregulated Serpine1 (PAI-1) gene expression by 2-6 fold in all groups except those supplemented with tPA. In the presence of tPA, TGF-β1 increased Serpine1 expression by as much as 57%, but this was not significantly different than untreated controls. The expression of Plau (tPA) was significantly downregulated 81% by TGF-β1 in the absence of Plasminogen and tPA (p<0.0001), but this effect was reduced and not significant in the presence of those two factors. Plau (uPA) expression was not significantly affected by TGF-β1, independent of plasminogen or tPA supplementation.

Discussion: In this study, we demonstrated that flexor tendon tenocytes treated with the pro-scarring growth factor, TGF-β1, had significantly reduced levels of active MMP-2 in the presence of plasminogen and tPA. The reduction in active MMP-2 was associated with reduced plasmin activity, reduced depletion of supplemented plasminogen, and an increase in total PAI-1 levels. These findings suggest that TGF-β1 reduces MMP-2 activity by suppressing the plasmin-mediated activation of MMP-2 via upregulation of PAI-1.

Significance: This data provides evidence that PAI-1 may be a novel therapeutic target for enhancing the activity of plasmin and MMP-2 during tendon healing. Therefore, PAI-1 inhibition may hold promise as a novel approach for the prevention of debilitating tendon adhesions and the promotion of scarless flexor tendon healing.

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