EFFECT OF STRESS DEPRIVATION ON MMP-1 GENE EXPRESSION AND REGULATION OF MMP-1 PROMOTER IN MEDIAL COLLATERAL AND ANTERIOR CRUCIATE LIGAMENTS (MCL, ACL) AND PATELLAR TENDON (PT).

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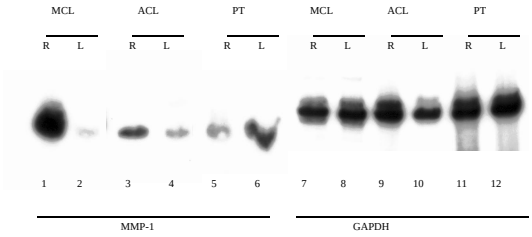
Relevance to Musculoskeletal Conditions: Stress deprivation leads to tissue degeneration, presumably due to a disfunction of the process of extracellular matrix (ECM) remodeling. Matrix metalloproteinase-1 (MMP-1), normally degrades collagen type I protein and is an important regulator of tissue remodeling during stress deprivation. We have determined changes in MMP-1 expression during stress deprivation and its transcriptional regulation in ligamentous cells.

Introduction: Considerable evidence from our laboratories suggests that regulation of ECM components in ligamentous and tendinous tissue are critically important to the process by which alterations in mechanical stress produces both structural and functional changes in periarticular connective tissue (PCT) (i.e. ACL, MCL, and PT). We have previously demonstrated a differential rate of collagen turnover in stress deprived PCTs[1]. Our published work has clearly demonstrated that all of the functional deficits are manifested by 12 weeks of immobilization in the rabbit. We have studied the changes in MMP-1 gene expression following 12 weeks of stress deprivation by joint immobilization and have isolated a portion of the MMP-1 promoter/enhancer region and studied the effect of this region on gene expression in cultured primary MCL cells.

Methods: Left knees of mature (8-10 mos old, 3.7 ± 0.2 kg) New Zealand white rabbits with closed epiphyses were immobilized for 12 wks in acute flexion (135°)[1].The right knee was used as the contralateral control. Rabbits were euthanized as per animal subjects protocol currently approved by UCSD-Office of Animal Resources and the mid portions of ACL, MCL and PT were isolated. The quantitative RT-PCR procedure was performed as previously described[2,3]. The mid-point of linear amplification was determined for 10^2, 10^3 and 10^4 template cDNAs using the same MMP-1, primer-pairs as those used for the experimental samples. The midpoint of linear amplification for 10^2, 10^3 and 10^4 MMP-1 templates was found to be 28 PCR cycles. Therefore the experimental determination was performed at 28 cycles. GAPDH message was amplified as an internal control to account for the differences in RNA extraction efficiencies. For promoter analysis study, MCL, cells were isolated and cultured as per our previously published protocols[4]. **MMP-1 promoter/enhancer constructs:** A portion of the MMP-1 promoter/enhancer sequence (-2968 to –3658) was placed downstream of a CAT gene cassette driven by the SV40 basal promoter. Transfection efficiency was normalized by co-transfection with a β-galactosidase gene driven by the human CMV promoter/enhancer containing expression vector. The parent vector containing CAT gene located downstream of the SV-40 basal promoter and lacking the MMP-1 enhancer sequences was transfected into control plates The plasmid DNAs were purified and transfected into MCL cells (Goomer et al. 1998b). The CAT gene expression was assessed after 60-72 hours. The data was normalized to beta-galactosidase protein expression for each plate transfected.

Results: MMP-1 gene expression analyzed by RT-PCR and quantitated by densitometric analysis and normalized to GAPDH message as relative ratio of MMP-1:GAPDH message and is shown as a histogram in figure 4. Comparison between left, stress deprived knee and right, contralateral control knee from same animals shows that MMP-1 message was decreased in stress deprived MCL by greater than 97% and increased in ACL and PT by 1000% and 200%, respectively (Figure 4: compare differences in relative levels of gene expression). The presence of the MMP-I specific promoter/enhancer fragment (-2968 to –3658) resulted in the activation of the CAT gene transcription by 22.4 ± 0.015% when compared with the basal promoter lacking this region of the MMP-1 promoter (figure 2: see histogram showing relative gene expression normalized to GAPDH).



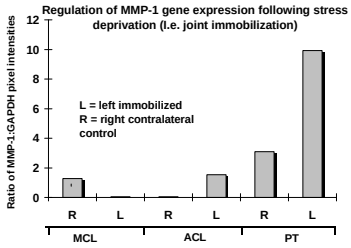


Figure 1: MMP-1 RNA expression following 12 weeks of stress deprivation in a rabbit model. A representative gel showing right (R) contralateral control and left (L) stress deprived tissues, MCL (lanes 1 and 2), ACL (lanes 3 and 4) and PT (lanes 5 and 6) assessed by quantitative RT-PCR. The midpoint of linear amplification for this prime-pair was determined to be 28 cycles, therefore, all PCRs were performed for 28 cycles. GAPDH mRNA expression was used as an internal control and assessed using the same conditions. Histogram (bottom) shows pixel intensity of the MMP-I (lanes 1-6) normalized to the GAPDH bands (lanes 7-12).

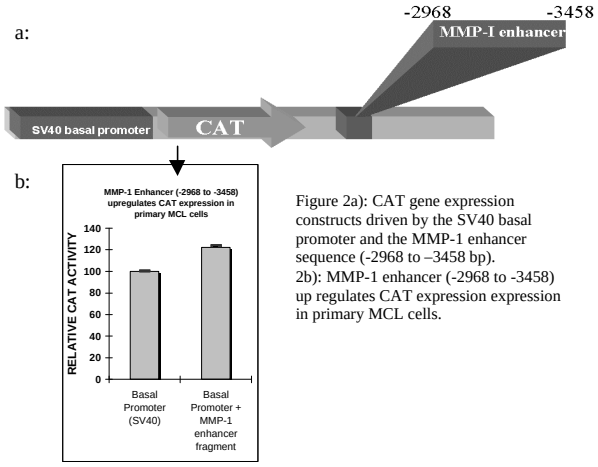


Figure 2a): CAT gene expression constructs driven by the SV40 basal promoter and the MMP-1 enhancer sequence (-2968 to –3458 bp).
2b): MMP-1 enhancer (-2968 to -3458) up regulates CAT expression expression in primary MCL cells.

Discussion In this study, we have demonstrated that stress deprivation by immobilization differentially effects MMP-1 gene expression in rabbit PCTs. This differential expression may explain the observed changes in collagen protein levels and tissue degeneration. These changes in gene expression are presumably transduced by the transcriptional regulation of the MMP-1 promoter/enhancer. A region of the promoter known to be involved in IL-1 induced expression of MMP-1 gene[5] was isolated and transcription directed by this region was analyzed in primary MCL cells in culture. Site-directed mutants of this enhancer fragment (fragment #1: -2968 to –3458) may, in the future, help elucidate consensus transcription factor binding sites that regulate MMP-1 gene expression as a function of stress-deprivation and/or mechanical strain.

References:1.Amiel et al. Clin. Orthop. 172:265:270;1983. 2. Goomer et al. Trans Orthop Res Soc 23(1):290; 1998. 3. Re et al. Analyt Biochem 225:1995. 4. Nagineni, et al. J. Orthop Res 10:465-475; 1992. 5. Vincenti et al. Arth and Rheum 29:574-582; 1996.

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