ADENOVIRUS MEDIATED GENE TRANSFER FOR TENDON HEALING

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Background:
Tears of the rotator cuff and Achilles tendon are a significant source of morbidity. There are few strategies available to enhance tendon healing. We have, however, found a small molecule known as nitric oxide to be implicated in a positive way in tendon healing. Under normal circumstances there is no, or very little, activity of the enzymes that synthesize nitric oxide in tendon. Once injured, however, there is a marked increase in the expression and activity of all three nitric oxide synthases (NOS) isoforms in rat Achilles tendon and human rotator cuff tendon cells. Inducible nitric oxide synthase (iNOS) is expressed early (in fibroblasts and macrophages), followed by endothelial NOS (eNOS) in endothelial cells and fibroblasts and bNOS in fibroblasts. We have also found that systemic inhibition of nitric oxide synthase inhibits the mechanical properties, collagen synthesis and cross-sectional area of healing rat Achilles tendon. Transcutaneous administration of NO enhances the healing of anal fissures in humans, but is poorly tolerated because of headaches from the systemic vasodilatory effects of NO. We wondered, therefore, if targeted gene therapy using adenovirus vectors might deliver NO to healing tendon and enhance the healing process with few side effects. As a first step in this process, we investigated the efficacy of gene transfer of adenovirus vectors containing the bacterial Lac Z gene in human fibroblasts in vitro and in a rat Achilles tendon healing model in vivo.

Methods:
For the in vitro work, human rotator cuff tendon samples were collected from surgery and cultured to 80% confluency. Cells were transfected for 2 hours with adenovirus containing the Lac Z, iNOS, eNOS, or bNOS gene and incubated for 24 hours for protein expression. mRNA expression for the three NOS isoforms was determined by RT-PCR. Protein expression was determined by X-gal staining and beta-galactosidase activity assay. For in vivo work, 12-week old male Sprague-Dawley rats were injected with 10^6 PFU adenoviruses. A dose response experiment was performed to determine the optimal transfection with varying concentrations of virus in vitro as well as a time course for protein expression of betagalactoside and nitric oxide synthase (using a ^3H L-arginine to ^3H L-citrulline assay).

Results:
Human rotator cuff tendon cells were successfully transfected with the Lac Z gene without impairing cell viability. Dose dependent experiments found 200 PFU adenoviruses was the optimal transfection dose. Both iNOS and eNOS could be successfully transfected to cultured human rotator cuff tendon cells using adenovirus constructs (Fig 1). Successful transfection of healing rat Achilles tendon was obtained with the Lac Z gene as determined by blue beta-gal staining of the healing tendon on day 4. Transfection with adenovirus containing the gene for iNOS resulted in a four-fold enhancement of NOS activity in healing rat Achilles tendon (Fig 2).

Conclusions:
These studies show that:
1) Adenovirus can be used to deliver a gene of interest to cultured human rotator cuff tendon in vitro.
2) Adenovirus can be used to deliver a gene of interest to healing tendon in vivo.
3) Transfection with the iNOS gene in vivo resulted in enhanced nitric oxide synthase activity in healing rat Achilles tendon.

Further studies are necessary to determine if NOS gene transfer can enhance tendon healing.

References:

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Figure 1: iNOS and eNOS mRNA expression in human rotator cuff tendon cells cultures by RT-PCR. MM: DNA molecular markers, Cells: untreated cells, Stim: TNF-α and LPS induced cells, Ad-E1 and E2: cells transfected with empty adenovirus, NOS1 and 2: cells transfected with adenovirus containing iNOS or eNOS gene. β-actin served as internal control. PCR product sizes are: iNOS~500bp, eNOS~471bp, β-actin~643bp.