ENHANCEMENT OF COLLAGEN SYNTHESIS IN HUMAN TENDON CELLS VIA iNOS GENE TRANSFECTION

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Introduction:
In many clinical situations, it is desired to increase collagen depositions as a means of enhancing wound strength and integrity. Both in vivo and in vitro, the link between the messenger molecule nitric oxide (NO) and collagen synthesis has been studied. Little is known, however, about the relationship between NO and collagen synthesis in tendon healing. The aim of this study was to investigate whether NO could enhance collagen synthesis in cultured human tendon cells via an adenovirus containing the gene for inducible nitric oxide synthase (Ad-iNOS).

Methods:
1. Adenovirus
Adenoviral constructs containing inserts of iNOS isoform gene, as well as the “empty” virus without insert, were purchased from the University of Iowa Gene Transfer Vector Core.

2. Tendon cell culture
Tendon cells from torn edge of the tendons of patients undergoing rotator cuff repair surgery were cultured following collagenase digestion. The cells were stimulated with exogenous NO donor (SNAP), transfected with Adenovirus, and/or treated with the NOS inhibitor, L-NMMA.

3. Assessment of cell viability
Cell viability was evaluated by MTS assay using CellTiter 96® Aqueous One Solution Cell Proliferation Assay kit (Promega).

4. Measurement of nitrite level
Nitrite (NO$_2^-$), the stable end product of nitric oxide, was measured in the culture media of cultured cells utilizing the spectrophotometric method based on the Greiss reaction.

5. Assay of collagen synthesis
Collagen synthesis for the tendon cells was evaluated by collagenase sensitive $[^{3}H]$-Proline incorporation ($[^{3}H]$).

Results:
1. Effects of NO donor on human tendon cells:
1.1 Cell viability
No significant effect on cell viability of human tendon cells was detectable following SNAP stimulation compared with control (cells only).

1.2 Nitrite production
Increasing doses of SNAP to treat the tendon cells resulted in a dose-dependent increase of nitrite release from the cells at 24 hours (Figure 1).

1.3 Collagen synthesis
Incubation of the tendon cells in the presence of 100 or 400 µM SNAP significantly increased absolutely collagen synthesis and compared to controls. Lower concentrations (10 and 50 µM) of SNAP had no effects on collagen synthesis and higher concentrations (800 µM) inhibited cellular collagen synthesis (Figure 2).

2. Effects of iNOS gene transfection on human tendon cells:
2.1 Cell viability
No significant effect on cell viability was detectable following iNOS gene transfection at 48 hours and treated with L-NMMA.

2.2 Nitrite production
After treated with Ad-iNOS and L-NMMA, the nitrite productions released from the tendon cells were shown in Figure 3.

2.3 Collagen synthesis
The tendon cells in the presence of 100 pfu/cell of Ad-iNOS significantly increased absolutely collagen synthesis (ACS) compared to Ad-Empty group. There was a significant inhibition effect of L-NMMA on collagen synthesis in 100 pfu/cell Ad-iNOS group (Figure 4).

Discussion and conclusion:
Our studies show for first time that nitric oxide can enhance collagen synthesis in human tendon cells in vitro. These results may explain, in part, at least, the beneficial effects of NO donors in tendonopathies in randomised clinical trials.

Reference:
2. Thornton FJ; Schaffer MR; Witte MB; Moldawer LL; MacKay SL; Abouhamze A; Biochem Biophys Res Commun 1998; 246: 654-9