

**Enhanced Cartilage Repair by Gene-Mediated Combination Insulin-Like Growth Factor-1/Interleukin-1 Receptor Antagonist Therapy—An In-Vitro Study**

**Summary:** This study examined the hypothesis that co-transduction of synovial lining cells with IGF-I and IL-1ra gene constructs would increase IGF-I and IL-1ra production and aid in the restoration of depleted cartilage. The data suggest that combination gene therapy using growth factors that stimulate cartilage matrix synthesis and IL-1ra to block IL-1 action have partial restorative effects on cartilage proteoglycan (PG) levels. However, PG levels in AdIGF-I and AdIGF-I/ AdIL-1ra combination therapy were similar, indicating the need for IL-1ra presence at the time of the IL-1 flux to prevent PG depletion, rather than after IL-1 actions had started.

Cartilage homeostasis relies on a balance between anabolic growth factors such as IGF-I and catabolic agents particularly IL-1. Catabolism predominates in disease conditions such as synovitis and osteoarthritis. From a therapeutic standpoint, IGF-I promotes cartilage healing when administered intraarticularly, but the residence time is limited to several days. Delivery of IGF-I by gene transduction of the synovial structures would be a less invasive and more persistent means of supplying growth factors. Abrogation of the catabolic axis through antagonism of IL-1 action would further add to the restoration of cartilage integrity, and combination of IL-1 receptor antagonist protein (IL-1ra) and IGF-I in the same gene construct, or given concurrently in stand-alone gene constructs, may provide dual axis therapy that can aid in cartilage resurfacing procedures and attenuate arthritic processes.

Drs. A. Nixon and J. Haupt from Cornell University worked with Colorado State University researchers Drs. S. Morisset and C. W. McIlwraith, as well as Dr. P. Robbins from University of Pittsburgh and Drs. C. Evans and S. Ghivizzani from Harvard Medical School, to examine the hypothesis that co-transduction of synovial lining cells with IGF-I and IL-1ra gene constructs would increase IGF-I and IL-1ra production and aid in the restoration of depleted cartilage.

Recombinant adenoviral vectors containing either the coding region of equine IGF-I (AdIGF-I) or the coding region of equine IL-1ra (AdIL-1ra) were constructed using the cre-lox system, and propagated in a 293 packaging cell line. Synovial membrane from 3 horses was digested in 0.15% collagenase and 0.015% DNaseI. Synovial monolayer cultures were established in 6-well plates and 8-well chamber slides and transduced with both AdIGF-I (200 moi), AdIL-1ra (100 moi), or both AdIGF-I and AdIL-1ra. Cultures were maintained for 4 days and medium harvested every 48 hours. Transduction was confirmed by Northern analysis, and levels of IGF-I

and IL-1ra in medium were determined by radioimmunoassay (RIA) or ELISA. The 8-well chamber slides were fixed in 4% paraformaldehyde, and processed for IGF-I and IL-1ra *in situ* hybridization and immunostaining.

A second experiment was then performed where synovial monolayers were transduced with AdIGF-I, AdIL-1ra, or both AdIGF-I and AdIL-1ra. IL-1 (10 ng/ml for 48 hours) depleted cartilage was exposed to the medium by synovial monolayer/cartilage explant co-culture. Medium was harvested every 48 hours, and explants and monolayers harvested after 6 days. Monolayer transduction was confirmed by Northern analysis, and the PG content of medium and explants assessed by DMMB dye-binding. The edge of explants was removed and fixed, paraffin embedded and sectioned for H&E and toluidine blue staining. Sections were also used for *in situ* localization of IGF-I, IL-1ra, and type II collagen gene expression.

Viral mediated IGF-I and IL-1ra transgene activity in monolayers was confirmed by Northern blot, with IGF-I and IL-1ra transcript bands of appropriate size (Fig 1).

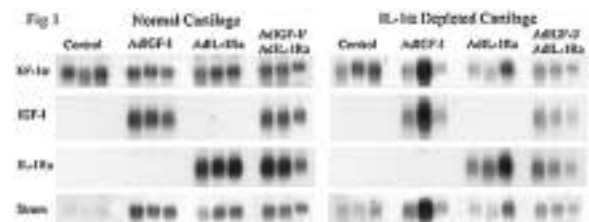


Figure 1.

Stromelysin expression was up-regulated by exposure to all adenoviral vector groups. Medium IGF-I was significantly elevated in all monolayer cultures transduced with AdIGF-I or cotransduced with AdIGF-I/AdIL-1ra (Table 1). IGF-I levels peaked on day 4 (110 ng/ml). Similarly, media IL-1ra concentrations were significantly elevated in

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AdIL-1ra and AdIGF/ IL-1ra co-infected synovial monolayers (Table 1), with the peak levels developing by day 2 (242 ng/ml).

Synovial Treatment	IGF-I Concentration (ng/mL)		IL-1Ra Concentration (ng/mL)	
	Normal Cartilage	IL-1 Depleted Cartilage	Normal Cartilage	IL-1 Depleted Cartilage
Control	0.004 "0.008a	0.356*0. 271a	0.004*0. 001a	0.005*0. 001a
AdIGF-I	45.62*4. 761b	86.09*14 .58b	0.0a	0.003*0. 001a
AdIL-1Ra	0.128*0. 065a	0.086*0. 030a	223.5 " 10.5 b	241.8 " 10.5 b
AdIGF-I/ AdIL-1Ra	65.21*10 .64 b	70.26*21 .15 b	213.3 " 10.5b	203.1 " 9.7b

Table 1. IGF-I and IL-1ra content of medium on day 2.

There was no significant difference in IL-1ra levels between AdIL-1ra or AdIGF-I/AdIL-1ra co-transduced cultures. Co-culture of transduced synovial monolayers with normal cartilage showed AdIGF-I and AdIGF-I/AdIL-1ra expressing synoviocytes increased PG content above uninfected controls (Fig 2). In IL-1 depleted cartilage explants AdIGF-I/AdIL-1ra cotransduction of synoviocytes improved PG content in cartilage, with AdIGF-I and AdIL-1ra alone producing lesser increases in PG level (Fig 3). Matrix proteoglycan staining on toluidine stained sections reflected the PG data from cartilage. (Fig 3)

Cells of the synovial lining were readily infected with both AdIGF-I and AdIL-1ra particles. Transduced cells secreted moderate levels of IGF-I and high levels of IL-1ra. The effect of this anabolic growth factor and catabolic blocker were most apparent in restoration of PG content of damaged cartilage in AdIGF-I and AdIGF-I/ AdIL-1ra co-cultures. These data suggest that combination gene therapy using growth factors that stimulate cartilage matrix synthesis and IL-1ra to block IL-1 action have partial restorative effects on cartilage PG levels. However, PG levels in AdIGF-I and AdIGF-I/ AdIL-1ra combination therapy were similar, indicating the need for IL-1ra presence at the time of the IL-1 flux to prevent PG depletion, rather than after IL-1 actions had started.

**Publications:**

Nixon AJ, Haupt JL, Frisbie DD, Morisset SS, McIlwraith CW, Robbins P, Ghivizzani S, Evans CA. "Enhanced cartilage repair by gene-mediated combination insulin-like growth factor-1/interleukin-1 receptor antagonist therapy." Proc Orthop Res Soc. 2002. 276.

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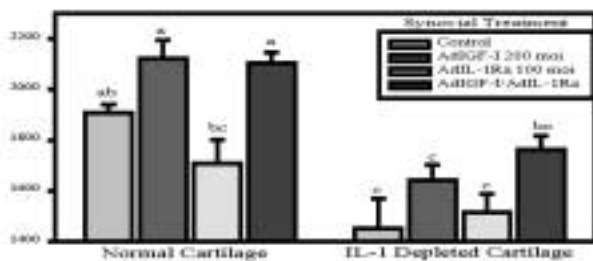


Figure 2.

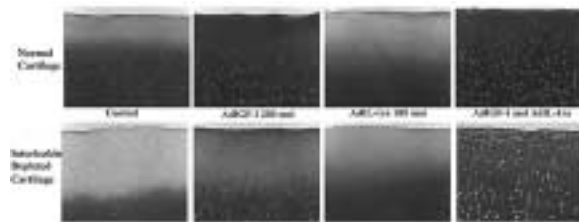


Figure 3.