A NEW GENE THERAPY APPROACH: IN VIVO TRANSFECTION OF ‘NAKED’ NFkB DECOY OLIGONUCLEOTIDE RESTORED DISC DEGENERATION IN THE RABBIT ANNULAR NEEDLE PUNCTURE MODEL

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INTRODUCTION: Degeneration of the intervertebral disc (IVD) is an important clinical problem that often contributes to low back pain. Progressive degradation of the extracellular matrix (ECM) has been implicated in the pathogenesis of IVD degeneration. Several pro-inflammatory cytokines (such as IL-1 and TNFα) and proteinases, which were detected in the degenerated disc, are thought to induce degradation of the ECM in the pathological IVD. Thus, the inhibition of cytokine and enzyme production by a gene therapy approach may provide an alternative strategy for the treatment of disc degeneration.

The transcription factor, nuclear factor kappaB (NFkB), is a heterodimeric DNA binding protein that plays a critical role in the regulation of many pro-inflammatory mediators. Recently, the transfection of decoy oligodeoxynucleotide (ODN) containing NFkB cis element has been reported as a feasible therapeutic approach for rheumatoid arthritis, osteoarthritis and cardiac diseases [1, 2, 3]. Therefore, we hypothesized that the injection of NFkB decoy ODN into the disc is effective in suppressing the gene expression of those pro-inflammatory cytokines and proteinases, thus delaying the progression of disc degeneration or inducing the restoration of the degenerated discs.

The purpose of this study was to evaluate the effect of single or double injections of “naked” decoy into the NP on the progression of disc degeneration in the rabbit annulus needle puncture model.

MATERIALS AND METHODS: Sequence target of NFkB decoy ODN: Phosphorothioate double-stranded decoy ODN against the NFkB binding site, for which sequences have been reported [3], was used (NFkB decoy ODN 5’-CCTGAGGATCCATCCTCC-3’ and 3’-GGAACTTCCCTAAAGGGAGG-5’).

Experimental Protocol: Twenty-four New Zealand White rabbits (3 kg) were used with IACUC approval. Under general anesthesia, rabbits received an annulus puncture in two non-contiguous lumbar IVDs (L2/3 and L4/5) with an 18G needle to a 5 mm depth to induce disc degeneration [4]. Discs at L3/4 served as non-punctured controls. These rabbits were equally divided into three groups: (punctured control, single-injection and double-injection groups). For the single-injection group, at the initial puncture, 1 µg or 10 µg of NFkB ODN decoy was injected into the punctured discs. Lateral X-rays of the lumbar spine were taken every two weeks to measure IVD height. Eight weeks after the first injection, lumbar spines were harvested and sagittal MRI images were taken.

Biodistribution of fluorescein-labeled decoy NFkB ODN: To examine the distribution of decoy ODN in vivo, FITC-labeled decoy ODN (10 µg) was injected into rabbit discs (L2/3 and L4/5) after annulus puncture as described above. On day-7 after the injection, under deep anesthesia, the rabbits were fixed, using a perfusion fixation technique. The animals were sacrificed and the IVDs were removed. Cryosections (6 µm) were cut, and the samples were imaged using confocal microscopy.

Radiographic Analysis: The IVD height was measured with a custom program that uses MathLab software and the percent DHI (% DHI = postoperative DHI/preoperative DHI x 100) was calculated.

MRI Analysis: Sagittal MRI of the lumbar spine at L2/3, L3/4 and L4/5 were assessed for MRI grade of disc degeneration using the MRI grading scale (0-3) as previously described [4].

Statistical Analysis: Differences among the groups were assessed for statistical significance by repeated ANOVA and the Fisher’s PLSD post hoc test. The Friedman test and Mann-Whitney U-test were applied for the MRI grading.

RESULTS: Distribution of FITC-labeled decoy ODN in vivo: On day-7 after injection, FITC-labeled decoy ODN was detected in both nucleus pulposus (NP) and annulus fibrosus (AF) tissues. Fluorescent intensity was confirmed in the nuclei and in the cytoplasm of the NP and AF cells (Fig. 1).

Change in DHI: The punctured group showed significant disc narrowing in the punctured discs two weeks after the puncture, which was maintained up to eight weeks (Fig. 2, pre-op vs. 2W, 4W, 6W, 8W, all p<0.01). In the single-injection group, there were no significant differences in the %DHI among the discs injected with 1 µg or 10 µg of decoy ODN and the punctured control discs at any time point (Fig. 2).

Four weeks after the initial surgery, in the double-injected animals the DHI of the 1 µg and 10 µg decoy ODN groups showed no significant differences from the punctured group, as was also seen in the single injection group (DHI at 4W, punctured: control 71.4±5.2%; 1 µg: 75.8±2.4%; 10 µg: 78.7±2.4%). However, after the second injection of decoy ODN at four weeks, the disc height began to recover, at six and eight weeks, towards the non-punctured control level in the discs injected with either 1 µg or 10 µg of decoy ODN. The recovery was significant in the 10 µg group 6W: 80.7±3.2%, p=0.07; 8W: 81.8±5.0%, p=0.12; 10 µg: 6W: 86.1±3.8%, p<0.01; 8W: 86.7±4.3%, p<0.05 vs. punctured control, Fig. 2).

MRI grade: MRI grading scores showed significant differences between the punctured group and the decoy-injected group (Control [puncture]: 2.1±0.6; single-injection [1 µg]: 1.4±0.7, p<0.01; single-injection [10 µg]: 1.7±0.7, p<0.05; double-injection [1 µg]: 1.8±0.7, p=0.21; double-injection (10 µg): 1.7±0.6, p<0.05 vs. control [puncture]).

DISCUSSION: For the first time, we present results showing that the intra-discal injection of ‘naked’ NFkB decoy ODN is effective in partially restoring disc height in the rabbit annular puncture model. Successful transfection of ‘naked’ decoy ODN into the NP and AF cells in vivo was confirmed by confocal image. The changes in disc height and MRI results provided strong evidence for the efficacy of this approach to achieve the structural restoration of the degenerated disc. Although the half-life of decoy ODN is not known, we hypothesize that a longer half-life is expected, due to the contained structure of the IVD. Further studies on the mechanism of action and studies in large animals should be performed.


ACKNOWLEDGMENT: This study was supported by a grant from AnGes MG Inc. and in part by NIH grants P50-AR39239 and P01-AR48152.

Fig. 1. Distribution of FITC-labeled decoy ODN in vivo (x 200)

Fig. 2. Change in %DHI