TissueGene-C (TG-C) Improves the Structural Recovery of Degenerated Discs in Rabbit Anular Puncture Model

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INTRODUCTION: Degenerative disc disease (DDD) is a common disorder that causes low back pain and dysfunction. Recent studies revealed that growth factors, such as transforming growth factor-beta 1 (TGF-β1), have positive effects on extracellular matrix (ECM) metabolism and cell proliferation in vitro and induce structural repair of intervertebral discs (IVDs) in various animal models [1,2]. TissueGene-C (TG-C) is a novel cell-mediated therapy comprised of allogenic non-transduced human chondrocytes and irradiated/transduced GP2-293 cells expressing TGF-β1 that has been shown to improve pain and reduce cartilage degeneration in patients with knee osteoarthritis [3,4]. Previous studies have shown that using TG-C led to strong analgesic effects in the rat monooiodoacetate-induced model of osteoarthritic pain by downregulating pain mediators and inhibiting neuronal sensitization [5]. To show the clinical utility of this cell therapy product to treat DDD, the effect of the intradiscal injection of TG-C on structure modification of disc degeneration needs to be confirmed in larger species. Using the rabbit anular puncture model, we hypothesize that an intradiscal injection of TG-C can inhibit or reverse chronic disc degeneration. The objective of this study was to compare radiographic and magnetic resonance imaging (MRI) changes between groups that received TG-C treatment and the untreated group to determine the efficacy of TG-C as a potential therapeutic intervention.

METHODS: All experiments were performed with IACUC approval of our institution.

Rabbit Anular Puncture Disc Degeneration Model [6]: Surgeries and Injection of TG-C: Under general anesthesia, lumbar IVDs of female New Zealand white rabbits (n=80, five months old) were exposed, and an anular puncture (18-gauge, 5 mm depth) was performed at two non-continuous discs (L2/3 and L4/5), with the disc (L3/4) between the punctured discs left intact as a control. Four weeks after the initial puncture, either the control (CS10; 10 µL per disc) or TG-C (Three doses Low; 1.5x10⁴, Mid; 5x10⁴, High; 1.5x10⁵; 10 µL per disc) was injected into the center of the nucleus pulposus (NP) using a fine tip 26-gauge needle (XX*MS16, Ito Corporation, Shizuoka, Japan) attached to a MS*GFN25 syringe (Ito Corporation). After 12 and 24 weeks, rabbits (10 per group) were sacrificed and subjected to radiographic and MRI analysis.

Radiographic analysis of disc height index (DHI): Lateral radiographs of the lumbar spine were obtained at two-week intervals up to 12 or 24 weeks after the initial puncture. IVD height was expressed as DHI, as previously described [7]. The average percent change in DHI of injected discs (both L2/3 and L4/5) was calculated for each postoperative disc as a ratio to its preoperative DHI (%DHI = (postoperative DHI/preoperative DHI) ×100) and further normalized to the DHI of the non-punctured disc (L3/4): [Normalized %DHI = (punctured DHI/non-punctured DHI) ×100]. All radiographs were assessed by an observer blinded to this experiment.

Magnetic resonance imaging (MRI) analysis: After sacrifice at 12 or 24 weeks, MRI examinations on isolated spine segments were performed using a Bruker BioSpec 3T scanner (BRUKER, Billerica, MA, USA). The average degeneration grade of injected discs (L2/3 and L4/5) was calculated according to Pfirrmann grade [8] using T2 weighted sagittal images; the evaluations were performed by two observers blinded to experimental groups.

Statistical analysis: The DHI was analyzed by two- or three-ways repeated ANOVA. The MRI grading data were assessed using Kruskal-Wallis Test with the Bonferroni correction.

RESULTS: Radiographic assessment of disc degeneration: Two-way ANOVA analysis for normalized %DHI, throughout the post-injection period, showed a significant improvement for all TG-C-injected groups over the CS10 control group (p<0.001). After 24 weeks of injections, all TG-C groups (Low, 84.6%, Mid, 86.6%, High 87.2%) showed significantly higher normalized % DHI than that of the CS10 group (69.6%, vs. all TG-C groups p<0.001) (Fig. 1). MRI assessment of disc degeneration grade (16 weeks): The Pfirrmann scoring, including both time points, showed a significantly low grade in the Mid and High TG-C groups, compared to the CS10 group (Three-way comparison, p<0.001, p<0.05, respectively, not shown in figures). The comparison for the 12-week group showed the same trends (Fig. 2a).

DISCUSSION: In rabbit anular puncture model, a TG-C intradiscal injection showed a significant recovery of IVD height and improved MRI scoring with a single injection. These results suggest that TG-C has the potential to induce structural modifications in degenerated discs.

SIGNIFICANCE/CLINICAL RELEVANCE: We have shown that a combination of allogenic non-transduced human chondrocytes and irradiated/transduced GP2-293 cells expressing TGF-β1 (TG-C) attenuated intervertebral disc degeneration in a rabbit anular puncture model. Therefore, the intradiscal injection of TG-C has potential as an effective therapeutic strategy for degenerated IVDs in human.


IMAGES AND TABLES: