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Injection of human umbilical tissue–derived cells into the nucleus pulposus alters the course of intervertebral disc degeneration in vivo

Steven K. Leckie, MD[a,*], Gwendolyn A. Sowa, MD, PhD[b], Bernard P. Bechara, MSc[b], Robert A. Hartman, MSc[a], Joao Paulo Coelho, MSc[b], William T. Witt, MSc[a], Qing D. Dong, MSc[a], Brent W. Bowman, BA[a], Kevin M. Bell, MSc[a], Nam V. Vo, PhD[a], Brian C. Kramer, PhD[c], James D. Kang, MD[a]

[a]*Department of Orthopedics, University of Pittsburgh Medical Center, 200 Lothrop St., Pittsburgh, PA 15213, USA*
[b]*Department of Physical Medicine & Rehabilitation, University of Pittsburgh Medical Center, 200 Lothrop St., Pittsburgh, PA 15213, USA*
[c]*Advanced Technology and Regenerative Medicine, Route 22 West, PO Box 151, Somerville, NJ 08876, USA*

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Abstract

BACKGROUND CONTEXT: Patients often present to spine clinic with evidence of intervertebral disc degeneration (IDD). If conservative management fails, a safe and effective injection directly into the disc might be preferable to the risks and morbidity of surgery.

PURPOSE: To determine whether injecting human umbilical tissue–derived cells (hUTC) into the nucleus pulposus (NP) might improve the course of IDD.

DESIGN: Prospective, randomized, blinded placebo–controlled in vivo study.

PATIENT SAMPLE: Skeletally mature New Zealand white rabbits.

OUTCOME MEASURES: Degree of IDD based on magnetic resonance imaging (MRI), biomechanics, and histology.

METHODS: Thirty skeletally mature New Zealand white rabbits were used in a previously validated rabbit annulotomy model for IDD. Discs L2–L3, L3–L4, and L4–L5 were surgically exposed and punctured to induce degeneration and then 3 weeks later the same discs were injected with hUTC with or without a hydrogel carrier. Serial MRIs obtained at 0, 3, 6, and 12 weeks were analyzed for evidence of degeneration qualitatively and quantitatively via NP area and MRI Index. The rabbits were sacrificed at 12 weeks and discs L4–L5 were analyzed histologically. The L3–L4 discs were fixed to a robotic arm and subjected to uniaxial compression, and viscoelastic displacement curves were generated.

RESULTS: Qualitatively, the MRIs demonstrated no evidence of degeneration in the control group over the course of 12 weeks. The punctured group yielded MRIs with the evidence of disc height loss and darkening, suggestive of degeneration. The three treatment groups (cells alone, carrier alone, or cells+carrier) generated MRIs with less qualitative evidence of degeneration than the punctured group. MRI Index and area for the cell and the cell+carrier groups were significantly distinct from the punctured group at 12 weeks. The carrier group generated MRI data that fell between control and punctured values but failed to reach a statistically significant difference from the punctured values. There were no statistically significant MRI differences among the three treatment groups. The treated groups also demonstrated viscoelastic properties that were distinct from the control and punctured values, with the cell curve more similar to the punctured curve and the carrier curve and carrier+cells curve more similar to the control curve (although no creep differences achieved statistical significance). There was some histological

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* Corresponding author. Department of Orthopedics, University of Pittsburgh Medical Center, 200 Lothrop St., Pittsburgh, PA 15213, USA. Tel.: (412) 648-1090; fax: (412) 383-5307.

E-mail address: leckiesk@upmc.edu (S.K. Leckie)

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evidence of improved cellularity and disc architecture in the treated discs compared with the punctured discs.
CONCLUSIONS: Treatment of degenerating rabbit intervertebral discs with hUTC in a hydrogel carrier solution might help restore the MRI, histological, and biomechanical properties toward those of nondegenerated controls. Treatment with cells in saline or a hydrogel carrier devoid of cells also might help restore some imaging, architectural, and physical properties to the degenerating disc. These data support the potential use of therapeutic cells in the treatment of disc degeneration.

Keywords: Intervertebral disc degeneration; Cellular therapy; Umbilical tissue–derived cells

Introduction

Intervertebral disc degeneration (IDD) is a frequently encountered condition by spine clinicians. In addition to adversely impacting quality of life, care for associated degenerative spinal pathology costs society over $40 billion annually in combined health-care spending and lost wages [1]. Patients who fail conservative management may become surgical candidates, although surgery does not reliably improve discogenic low back pain [2,3] and may induce adjacent segment degeneration [4]. The intervertebral disc (IVD) is a biologically harsh environment [5] and has a poor intrinsic healing capability [6]. Less invasive treatment paradigms that can safely and effectively alter the course of disc degeneration without hindering motion could have a significant impact on the lives of many patients.

Alternative biological treatments, such as growth factors, cell transplantation, and gene therapy [7,8], are under active investigation. Intervertebral disc degeneration is a complex process involving desiccation and loss of cellularity and extracellular matrix within the nucleus pulposus (NP) [9,10], and the rationale for cell-based therapy is to repopulate the disc so that the cells can renew synthesis of extracellular matrix and regenerate a biologically active environment. Cultured human amniocytes can be induced to differentiate into ectodermal and mesodermal cell types in vitro (including neuronal and osteogenic cells) even after several decades of cryopreservation [11]. In addition to cells found in the amniotic fluid, research has also focused on cells derived from Wharton's jelly [12], umbilical cord blood [13], and the placenta [14,15]. Human postpartum umbilical cord tissue is an attractive cell source because the cells are easily harvested from tissue that is abundantly available and otherwise discarded, as it is generated with every childbirth. Donor cords are easily collected without putting the donor at risk, and the cells do not carry the ethical conflicts that are associated with embryonic stem cells or fetal cells. Another advantage to human umbilical tissue–derived cells (hUTC) is that they can be manufactured from a single donor under Good Manufacturing Process regulations to create large cell banks that may be used as needed. Human umbilical tissue–derived cells have recently been shown to secrete brain derived neurotrophic factor (a nerve growth factor) in vitro [16] and to reduce neurologic deficits in a rodent model of stroke after intravenous administration [17]. These studies demonstrate that transplanted hUTC are able to elicit a biological response.

Hydrogel NP replacement technology is also an active area of investigation. As the disc degenerates, the loss of disc height leads to an increased pressure on the facets and altered mechanical properties [18]. Hydrogels injected into the NP space can help restore the disc height (thereby normalizing biomechanical properties), and provide a scaffold for native cells to repopulate the disc and regenerate extracellular matrix [19,20].

In this study we investigate the utility of injecting hUTC directly into the NP in a surgical in vivo model of IDD. The hUTC were injected with and without a hydrogel carrier. This study is among the first to quantify the magnetic resonance imaging (MRI) and biomechanical responses to treatment in this degeneration model. We hypothesize that treating the degenerating IVD with either hUTC or a hydrogel carrier will slow the course of degeneration and that treating the disc with a combination of cells in a hydrogel carrier will have a more salutary effect than either intervention alone.

Methods

Rabbits

Thirty healthy skeletally mature New Zealand white rabbits were used in this study (female; age, 1 year; weight, 5 kg). They were split into nonpunctured control (n=6 rabbits, 18 discs), puncture (n=6 rabbits, 18 discs), puncture followed by injection of the carrier alone (n=6 rabbits, 18 discs), puncture followed by the injection of hUTC in phosphate-buffered saline (PBS) buffer (n=6 rabbits, 18 discs), and puncture followed by the injection of hUTC in a carrier (n=6 rabbits, 18 discs) (Table). The number of rabbits per group was selected based on our previous experience with this model and is typical for studies of this nature [7,8,21–27]. This investigation was performed in full compliance with the Institutional Animal Care and Use Committee at our institution (protocols 0901919 and 0809294).

Sample preparation

Human umbilical cord tissue was obtained from a consented donor undergoing either vaginal or cesarean