Video Article

Ovine Lumbar Intervertebral Disc Degeneration Model Utilizing a Lateral Retroperitoneal Drill Bit Injury

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Abstract

Intervertebral disc degeneration is a significant contributor to the development of back pain and the leading cause of disability worldwide. Numerous animal models of intervertebral disc degeneration have been developed. The ideal animal model should closely mimic the human intervertebral disc with regard to morphology, biomechanical properties and the absence of notochordal cells. The sheep lumbar intervertebral disc model fulfils these criteria. We present an ovine model of intervertebral disc degeneration utilizing a drill bit injury through a lateral retroperitoneal approach. The lateral approach significantly reduces the incision and potential morbidity associated with the traditional anterior approach to the ovine spine. Utilization of a drill-bit method of injury affords the ability to produce a consistent and reproducible injury, of precise dimensions, that initiates a consistent degree of intervertebral disc degeneration. The focal nature of the annular and nucleus pulposus defect more closely mimics the clinical condition of focal intervertebral disc herniation. Sheep recover rapidly following this procedure and are typically mobile and eating within the hour. Intervertebral disc degeneration ensues and sheep undergo necropsy and subsequent analysis at periods from eight weeks. We believe that the drill bit injury model of intervertebral disc degeneration offers advantages over more conventional annular injury models.

Video Link

The video component of this article can be found at https://www.jove.com/video/55753/

Introduction

Lower back pain is the leading cause of disability worldwide[1]. Lumbar intervertebral disc degeneration associated discogenic pain is considered a significant contributor to lower back pain[2]. There is an increasing demand for reliable animal models of intervertebral disc disease for broadening the understanding of the degenerative process and for the investigation of potential therapies.

Numerous animal models of intervertebral disc degeneration exist[3]. Animals models used in the investigation of degenerative disc disease range in size from mice[4], to larger mammals such as dogs[5], sheep[6], and non-human primates[7]. Methods used to induce intervertebral disc degeneration can be broadly classified into the categories of mechanical (*e.g.* intervertebral disc compression[8] or surgical injury[6]), chemical (*e.g.* chemical nucleolysis[5]) or, less commonly, spontaneous degeneration (*e.g.* the sand rat[9]).

Given the complexity of human intervertebral disc degeneration, a perfect animal model does not exist. However, important considerations in choosing an appropriate animal model to mimic this condition closely have been identified[3]. Such considerations include the absence of notochordal cells (primitive cells with possible progenitor cell function[10] absent from the adult nucleus pulposus in humans, sheep, goats and chondrodystrophic dogs but present in most mammals), similarities in animal and intervertebral disc size relative to humans, comparable biomechanical forces to the clinical condition, mechanistic and ethical considerations[3].

Non-human primates meet many of the above criteria. Baboon and macaque models of spontaneous intervertebral disc degeneration have been described[11,12,13]. Both species spend large amounts of time in erect or semi-erect postures — a distinct advantage relative to other animal models. However, ethical and practical consideration (*e.g.* expense, housing, delayed onset of spontaneous degeneration) restrict their use in many institutions.

The ovine spine is an established model of intervertebral disc degeneration, with advantages including cellular, biomechanical and anatomical similarities to the human spine[10,14,15]. Despite the quadrupedal stature of sheep the ovine lumbar intervertebral disc is exposed to similar stresses to the human disc[14]. The ovine model is also more widely accepted, from an ethical perspective, than non-human primate models. Varied methods have been described to initiate the degenerative process, many of which require direct access to the intervertebral disc. Due to the termination of the spinal cord in the sacral region and ossification of the posterior longitudinal ligament in the ovine lumbar spine, posterior approaches to the intervertebral disc are technically challenging and less commonly used in the sheep[16]. The traditional access routes to the sheep lumbar spine, *i.e.* via anterior or anterolateral approaches, require large abdominal incisions, are fraught with risks of hernia, and damage to internal viscera and neurovascular structures[16]. The use of a relatively small lateral incision away from dependent abdominal areas may decrease such risks[17].

We present an ovine model of degenerative lumbar intervertebral disc disease using drill bit injury performed through a minimally invasive lateral approach, and inspired by the work of Zhang *et. al*[18]. The goal of this protocol is to enable a reliable lumbar disc injury model that is readily reproducible, produces a consistent injury, and is safe and well tolerated. This approach is well-suited to investigators seeking to induce a milder degree of lumbar intervertebral disc degeneration than that observed with traditional surgical annulotomy (unpublished data) for the investigation of either intervertebral disc degeneration or regenerative therapies. These findings will be described in a forthcoming publication.

Protocol

The protocol detailed in this manuscript follows the animal care guidelines of Monash University Animal Ethics. Animal ethics approval for this protocol has been granted by Monash University Animal Ethics. Ethics approval number: MMCA/2014/55

1. Sheep Preparation

NOTE: Ewes aged two to four years were used.

1. Fast sheep for 18 h prior to anesthesia. Provide animals with access to water until 6-12 h prior to operation[19].
2. Sedate animals by intravenous injection of medetomidine hydrochloride (0.015-0.020 mg/kg) to facilitate transfer to operating suite.
   NOTE: The medetomidine hydrochloride serves to reduce animal stress and agitation associated with separation from other animals for transfer to the operating suite.
3. Inject thiopentone (10-13 mL/kg) for induction of anesthesia upon arrival to the operating suite.
4. Administer prophylactic intravenous antibiotics (amoxicillin 1 g IV) immediately following thiopentone injection.
5. Intubate sheep using a size 7.5-9 mm (internal diameter) endotracheal tube[20].
6. Maintain anesthesia using inhaled isoflurane (2-3%) in 100% oxygen at a flow rate of 2 L/min. Attach a pulse oximeter to the sheep's ear.
7. Closely monitor the sheep's vital signs (heart rate, respiratory rate and oxygen saturation via pulse oximeter and observation) and level of consciousness.
   NOTE: Indicators of light anesthesia such as spontaneous chewing, active regurgitation, and spontaneous movements should prompt increase in the level of anesthesia. Red flag signs indicating urgent lightening of anesthesia include respiratory compromise and severe bradycardia. Rotation of the eye is not a consistent indicator of depth of anesthesia in sheep[19].

2. Disc Level and Incision

1. Collect the surgical tools needed for this procedure: veterinary clippers, 20 mL luer-lock syringe, 21G IV Needle, #4 scalpel handle, #22 scalpel blades, Gillies tissue forceps, Metzenbaum curved dissecting scissors, Deaver retractor, Hohmann retractor blade, 3.5 mm Brad point drill bit, drill bit stop, drill, autoclavable veterinary drill bag, needle holder, 2-0 absorbable synthetic braided sutures, 3-0 absorbable synthetic braided suture and Mayo suture scissors.
2. Prepare the operating suite. Clean the operative table and instrument stand with 70% ethanol. Autoclave all surgical instruments prior to operation. Perform pre-operative anesthetic check.
3. Place the sheep on the operating table in the right lateral position.
4. Using electronic clippers, shave the region defined superiorly by the lower ribs, inferiorly by the iliac bone, medially by the contralateral lumbar transverse processes and approximately 10 cm lateral to the ipsilateral lumbar transverse processes.
5. Palpate the iliac crest, lumbar transverse processes (L1-6) and costo-vertebral angle for the landmarks for surgical incision site. Mark these landmarks with a sterile pen.
6. Prepare the lateral abdomen by disinfecting with chlorhexidine and alcoholic-iodide antiseptic wash.
7. **Observe standard surgical aseptic techniques throughout the operation. The surgical team scrubs prior to the operation. Place a sterile fenestrated square drape over the surgical site, and a large sterile square drape on the overhead table.**
   1. Sterilize all items to be used within the operative site prior to the operation. Monitor and maintain sterility of the surgical site throughout the operation. Ensure all items introduced into the sterile field are sterile and transferred in a sterile fashion.
8. Use surgical loupe magnification and a headlight to facilitate visualization during the surgical procedure.
9. Make a longitudinal incision using the #22 scalpel blade attached to the #4 scalpel handle parallel to and 1 cm anterior to one to two lumbar transverse processes above and below the intervertebral disc levels of interest.
   NOTE: Further information regarding incision planning can be found in the discussion.
10. Use the monopolar diathermy to divide the underlying subcutaneous tissue and the lateral aspect of the abdominal wall musculature; direct the dissection towards the tips of the lumbar transverse processes above and below the intervertebral discs of interest.
11. Divide the thoracolumbar fascia longitudinally at its attachment to the transverse processes.

12. Visualize and preserve the underlying *quadratus lumborum*, psoas muscles and the traversing neurovascular bundles.
13. Maintain hemostasis through the procedure using diathermy.
14. Sweep the fingers between the plane of the peritoneum and posterior abdominal wall musculature at the exposed intervertebral disc levels to perform digital blunt dissection.
15. Retract the *quadratus lumborum* and psoas muscles posterolaterally using a Deaver retractor to further expose of the intervertebral discs.
16. Palpate for the concave intervertebral bodies and the convex intervertebral discs.
17. Position the retractors immediately over the discs and take care to ensure lumbar vessels are not damaged.
18. Using surgical loupe magnification with a headlight, identify the lumbar vessels which are located approximately 1 cm caudal to the inferior endplate.
19. Perform an intraoperative lateral X-ray to confirm the disc level.[21]
    Note: Radiograph settings: 47kV; 4 mAs[21]
20. **Depending on the disc levels desired, expose the intervertebral disc by separating the surrounding structures and attachments as below.**
    1. For levels L3/4 and above, sweep aside the muscular attachments over the disc using digital blunt dissections.
    2. For levels L4/5 and below, sharply divide the thicker tendinous muscular attachments over the disc using bipolar diathermy and scissors.
       NOTE: L6/S1 disc can be difficult to access due to obstruction by the iliac crest. If access cannot be accomplished via the lateral approach an anterior approach may be utilized.

3. Drill Bit Injury

NOTE: Pre-operative planning includes the allocation of injury/treatment levels and control levels. Further information regarding level allocation can be found in the discussion.

1. Define the drill bit entry point by observing the left lateral and anterior extremities of the intervertebral disc.
   NOTE: The entry point is located at the midpoint of this left anterolateral quadrant (defined by the anterior and lateral extremities of the disc). The drill bit is inserted at this entry point with a trajectory aimed towards the center of the intervertebral discs and directed slightly cranial to perpendicular.
2. **Fit a Brad-point drill bit into the power drill. Ensure the diameter of the drill bit is slightly less than the intervertebral disc height *i.e.* ~3.5 mm for lumbar intervertebral disc in 60-70 kg sheep.**
    1. Apply a drill bit stop to provide an unprotected drill bit length of approximately half the diameter of the lumbar intervertebral disc *i.e.* ~12 mm for lumbar intervertebral discs in 60-70 kg sheep.

3. Apply the drill bit to the entry point and direct it in a trajectory slightly cranial to the center of the intervertebral disc. The slight cranial angulation is to minimize the risk of endplate injury.
4. Advance the drill bit slowly into the intervertebral disc with the drill on low power for 1 s. Adjust the trajectory in a slight cranial or caudal fashion if excessive resistance is encountered
   NOTE: Such excessive resistance likely indicates contact with the endplate.

4. Closure

1. Once hemostasis is achieved, irrigate the wound with Ringers' solution.
2. Perform layered closure, preferably using 2-0 absorbable synthetic braided sutures to the lateral abdominal wall tissues and continuous 3-0 undyed absorbable synthetic braided subcuticular suture to the skin.

5. Post-operative Management

1. Place a fentanyl transdermal patch (75 µg/h) in the inguinal region for post-surgical analgesia for 3 days.
2. Additionally, use intravenous buprenorphine (0.005-0.01 mg/kg) for top-up analgesia if needed.
3. Cease the inhalational anesthetic. When spontaneous breathing occurs, remove the endotracheal tube.
4. Allow the animal to recover in a holding cage under constant observation.
   NOTE: The animal should not be left unattended until it has regained sufficient consciousness to retain sternal recumbency.
5. Once the animal is fully alert and standing, re-introduce food and water. Once fully recovered, return the animal to its operative facility holding pen with other animals.
6. Monitor closely for 24 h and continue observation for one week. Monitor for evidence of post-surgical pain or distress.
   NOTE: Post-operative transdermal fentanyl patch applied for three days should provide sufficient analgesia. Additional analgesic requirements should prompt animal review.
7. Feed the sheep normally, and allow the sheep to carry out normal activities without restriction. Observe the sheep for any evidence of neurological deficit such as lameness.
   NOTE: The intervertebral disc defect produced by the drill bit injury method is on the anterolateral aspect of the disc and the injury depth is limited by the drill bit stop to the mid nucleus. As the neural elements are located posterior/posterolateral to the intervertebral disc, the risk of neural compromise secondary to symptomatic nucleus pulposus is remote. This anatomical characteristic of the model precludes the use of neurological examination to distinguish intervertebral disc degeneration with and without nucleus pulposus herniation.
8. Return the sheep to the university farm to await euthanasia and necropsy at the end of the experimental period.

6. Euthanasia

1. Perform sheep euthanasia at an appropriate time interval following drill bit intervertebral disc injury.
2. Inject intravenous pentobarbitone sodium (>100 mg/kg) for euthanasia.

Representative Results

Pre-operatively, sheep underwent baseline 3T magnetic resonance imaging (MRI) for assessment of underlying intervertebral disc morphology and degeneration. Sheep underwent additional intra-operative lateral radiography for confirmation of intervertebral disc level and calculation of disc height index. A pre-operative sagittal plane slice from 3T MRI and an intra-operative radiograph are demonstrated in **Figure 1**.

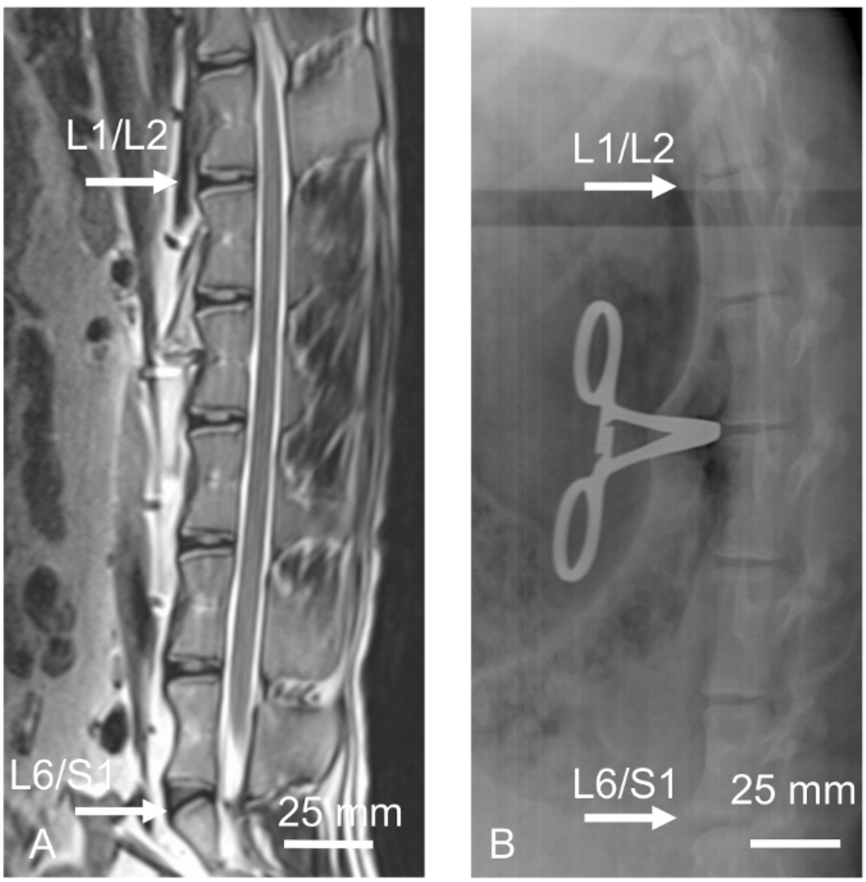

Figure 1: Pre-operative 3T MRI (**A**) and Intra-operative Lateral Radiograph (**B**). (**A**) Sagittal slice from 3T MRI (3T T2-weighted spin echo sequence) of ovine lumbar spine demonstrating lumbar 1/2 (L1/2) to lumbosacral (L6/S1) intervertebral discs. Intervertebral discs have a homogenous hyperintense appearance indicating no evidence of significant pre-operative intervertebral disc degeneration. Note that the ovine lumbar spine normally has six lumbar vertebrae, and the ovine spinal cord terminates in the sacral region. (**B**) Intra-operative lateral radiograph (settings: 47 kV; 4 mAs) demonstrating L1/L2 and L6/S1 intervertebral discs with the surgical instrument marking the L3/L4 intervertebral disc. Scale bars = 25 mm. Please click here to view a larger version of this figure.

Following the surgery, sheep typically recovered and were independently mobile within 1 h. Sheep were observed closely for one week, and subsequently returned to farmland until necropsy at 8 weeks following intervertebral disc injury. No adverse events occurred. At 8 weeks following disc injury, sheep underwent necropsy, X-ray and MRI of lumbar spines, and processing of discs for histological and biochemical analysis.

Representative post-operative images of the gross morphological appearance, and radiological 9.4T MRI images of injured sheep lumbar intervertebral discs at 8 weeks (56 days) post injury are shown in **Figure 2**. The gross morphological image demonstrates the drill bit injury tract penetrating the annulus fibrosus and extending into the nucleus pulposus. This is also evident in the 9.4T MRI. Comprehensive description and analysis of the outcome of this approach will be described in a forthcoming publication detailing the model validation study.

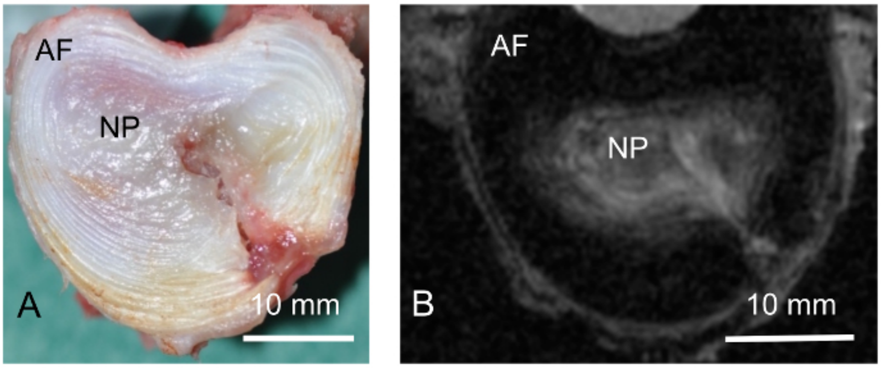

Figure 2: Gross Morphological and MRI Images of Injured Disc. (**A**). Gross morphological image of intervertebral disc demonstrating injury tract penetrating annulus fibrosus (AF) and extending into nucleus pulposus (NP). (**B**). 9.4T MRI (T2-weighted fast spin echo sequence) also demonstrating injury tract penetrating through AF into NP. Scale bar = 10 mm. Please click here to view a larger version of this figure.

Discussion

This minimally invasive lateral access approach is efficacious and safe with no post-operative herniae, abdominal wound dehiscence or infection observed in this series. Use of the drill bit intervertebral disc injury model with a depth stop provides a reproducible method of inducing a consistent intervertebral disc injury of known dimension (*i.e.* a 3.5 mm diameter x 12 mm depth injury in this study). In our experience, this method produces a less severe degree of disc degeneration than that observed in conventionally described ovine scalpel blade lumbar intervertebral disc annulotomy models[6,22] (unpublished data). This will be described in a forthcoming publication.

In making the initial longitudinal skin incision (step 2.9), the exact length and location of the incision should be modified based on the desired disc levels. More superior disc levels (T12/L1) can be reached by extending the incision to the costovertebral angle, whilst an incision extending to the iliac crest will allow access to the lower lumbar spine (to L5/L6). A 10 cm cut will facilitate access to three to four disc levels, while a smaller focused incision at 5 cm is necessary for access to single-discs. We prefer to perform injury at two levels, usually L2/L3 and L3/4. This enables the adjacent L1/2 and L4/5 intervertebral disc levels to be utilized as non-injured internal controls. Once technically confident, the surgical procedure on one sheep can be completed in less than one hour with minimal blood loss and discomfort[18]. The critical step and major technical challenge of this technique is the avoidance of endplate injury during drill bit disc injury. Clearly defining the superior and inferior margins of the intervertebral disc at the entry point of the drill-bit, is of the utmost importance. Slowly progressing the drill on low speed into the intervertebral disc, starting approximately perpendicular with slight cranial angulation also minimizes the risk of endplate injury. Lengthening of the skin incision may be required to obtain sufficient angulation of the drill.

Simple modifications to this technique include changes in drill bit size and depth, as these will be dictated by the size of the animal and lumbar intervertebral discs. This approach can be used to reliably induce degeneration in the intervertebral discs from T12/L1 to L5/6. The retroperitoneal approach may be used to access the intervertebral disc to induce degeneration by other mechanisms[16] or administer experimental therapeutic agents.

Limitations of this approach relate to the extent of the intervertebral disc injury and subsequent degeneration induced by this approach. If an investigator seeks to induce severe intervertebral disc degeneration, other more aggressive methods of disc injury such as scalpel blade annulotomy[6] should be considered. The acute defect produced in the intervertebral disc by the drill bit method of injury is relatively small, and may not be well suited to the administration of therapeutics at the time of injury.

The ovine spine was chosen for the intervertebral disc injury model for several reasons. Non-human primates, despite their anatomical and biomechanical similarities to the clinical condition (*i.e.* large amounts of time in erect and semi-erect postures), present sufficient ethical and practical considerations to prevent their utilization in many institutions. Although a quadruped, the sheep lumbar intervertebral disc is anatomically comparable and exposed to similar biomechanical stresses to human lumbar intervertebral disc[16,18]. Sheep demonstrate the loss of notochordal cells from the nucleus pulposus in early adulthood, as do humans[10,23]. Notochordal cells may have progenitor cell function and have been demonstrated to influence the course of disc degeneration through regeneration of the disc matrix. Finally, from a pragmatic perspective, sheep are hardy animals able to tolerate surgery well, are readily available, and present an economically feasible option[16,18].

The goat[18] is another animal model of lumbar disc degeneration that presents many of the advantages of the sheep model – similar size, economic feasibility, resilience, and absence of notochordal cells in the adult[24]. Other large animal models present additional challenges - the presence of notochordal cells in the porcine model, and ethical issues that may be associated with canine models. For a comprehensive review of animal models of intervertebral disc degeneration, the reader is directed to a recent review by Daly *et. al*[3].

As the ovine intervertebral disc demonstrates spontaneous loss of notochordal cells and undergo progressive degeneration with age[23], it is imperative to ensure consistency of sheep age in experiments. We prefer to use ewes aged two to four years, as at this age, notochordal cells are now absent[23]. From our own experience, minimal spontaneous degeneration has occurred in sheep aged from two to four years despite the loss of notochordal cells. Furthermore, the sheep vertebral body growth plate closes at approximately 24 months with vertebral body growth having ceased months earlier[25], minimizing the risk of any influence on disc regeneration from adjacent growth plate cells. Ewes were preferred because they are less aggressive than their male counterparts facilitating easier animal handling. If male sheep are used, we recommend using wethers.

In a study by Zhang[18] using a similar method of drill bit injury, where a drill bit measuring 4.5 mm in diameter was inserted 15 mm deep with manual rotation of 360° to produce disc degeneration in goats, there was no statistically significant difference in radiographic Pfirrmann degenerative score in the injured discs as compared to preoperative images. There was, however, demonstrable histological evidence of mild to moderate disc degeneration[26]. In contrast in this study, gross morphological and 9.4T MRI analysis revealed evidence of significant degenerative changes in the lumbar intervertebral discs, indicating the significant advantage of this approach.

The application and outcome of this method will be described in a forthcoming publication comparing the drill bit method of intervertebral disc injury to the established annulotomy method in the ovine model. This method may also be used in future for the investigation of regenerative therapies.

Disclosures

The authors declare they have no competing financial interests to disclose.

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