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Technical Report

Retroperitoneal approach to the intervertebral disc for the annular puncture model of intervertebral disc degeneration in the rabbit

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Abstract

BACKGROUND CONTEXT: The rabbit annular puncture model of degeneration is among the most widely used models of intervertebral disc (IVD) degeneration. There are no published reports of the specific surgical technique used to produce this model.

PURPOSE: To describe the model in detail in an effort to reduce center-to-center variability and hopefully improve the reproducibility of future experimental results.

STUDY DESIGN: Technical report of surgical approach and experience.

PATIENT SAMPLE: New Zealand White Rabbits.

METHODS: A detailed report of the annular puncture technique in rabbits is provided including preparation of the animals, instrumentation, a description of retroperitoneal approach to the lumbar area, and the technique for IVD injury. Common pitfalls and complications of the procedure are described.

CONCLUSIONS: The methods described can serve to standardize the implementation of this important preclinical model of disc degeneration.

Keywords: Rabbit model; Intervertebral disc degeneration; Annular puncture; Retroperitoneal approach

Introduction

Despite its common use, there has been no detailed report of the specific surgical technique used in the annular puncture model of intervertebral disc (IVD) degeneration in rabbits. The previously published descriptions of the model demonstrate its efficacy in producing degenerative changes of the IVD but do not describe the exact steps and equipment necessary for implementation. Thus, there may be discrepancies between techniques used that could ultimately affect the ability to effectively compare results from different centers using this model [1]. The following technical report serves to describe the model in detail in an effort to reduce center-to-center variability and improve the reproducibility of future experimental results.

FDA device/drug status: Not applicable.

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Preclinical animal models of disease are an important step in the translational development of novel biomedical technologies. For maximal utility, an animal model not only needs to adequately model the human disease process under investigation but also be economically feasible, conform to ethics guidelines, and be reproducible [2]. The literature contains reports of several experimentally induced models of IVD degeneration [3–14]. Injury to the annulus fibrosus and subsequent nucleus pulposus denucleation is a common method used to produce IVD degeneration in many animals as it is both easy to perform and reliable. Masuda et al. [15] and Sobajima et al. [13] developed and reported on the rabbit annular puncture model in which an 18G needle is used to create an initial injury that produces slow progressive degenerative changes in the IVD over a period of 4 to 8 weeks. The slow development of IVD degeneration induced with this model is similar to that seen in human disease and thus better suited to test novel therapies. This was a significant innovation over previous models that used a scalpel to produce a more rapid and severe course of degeneration [16]. Furthermore, rabbits are the smallest animals in which this type of disc injury is possible without using more sophisticated microsurgical techniques. For these reasons, along with the relatively modest cost for the purchase and housing of rabbits, the rabbit annular puncture model has become the most widely used model of IVD degeneration [17–24].

Methods

Animal preparation

After approval from the institutional animal care committee, New Zealand White rabbits, generally 2.7 to 3.2 kg correlating to an age of approximately 3 months, are purchased (Myrtle's Rabbitry, Inc., Thompsons Station, TN, USA) and allowed to acclimatize to the local environment for at least 1 week. The animal is given preemptive analgesics (meloxicam 0.2 mg/kg; Boehringer Ingelheim, Ridgefield, CT, USA) by subcutaneous administration the day before the procedure. The rabbit is tranquilized by subcutaneous injection of ketamine hydrochloride (40 mg/kg; Putney, Inc., Portland, ME, USA) and acepromazine hydrochloride (1 mg/kg; Phoenix Pharmaceuticals, Inc., St Joseph, MO, USA). A preoperative lateral radiograph is taken of the lumbar spine to serve as a baseline for future comparison and determine the spinal anatomy to allow for appropriate IVD-level selection. Immediately afterward, anesthesia is supplemented with xylazine (5 mg/kg, Lloyd Laboratories, Shenandoah, IA, USA) given subcutaneously. The animal is endotracheally intubated and placed on isoflurane gas (induction 1.5–2.5%, maintenance 1–2%; Webster Veterinary, Devens, MA, USA) anesthesia in oxygen. Buprenorphine analgesic (0.03 mg/kg; Reckitt Benckiser Pharmaceuticals, Richmond, VA, USA) is given subcutaneously. The left flank is then shaved from ventral to dorsal midlines and from the twelfth rib to the iliac crest. The rabbit is then relocated to the operating suite and positioned in the left lateral decubitus position. The skin is prepared for aseptic surgery via a triple wash/rinse with povidone iodine scrub and alcohol rinse. The animal is draped to isolate the prepped area. Isoflurane by inhalation is used to maintain surgical depth anesthesia throughout the procedure.

Instruments needed

Instruments needed include no. 15 scalpel; 18G needle with cap trimmed to depth of 5 mm [15]; Miller-Senn retractors; small S-shaped double-ended retractors (Medline industries MDS1817612, Mundelein, IL, USA); Adson forceps; small hemostats; titanium vascular clips (Auto Suture Premium Surgiclip II; Coviden, Mansfield, MA, USA); and 4×4 gauze sponges (Fig. 1).

Exposure of the IVD

The most anatomic exposure of the lumbar spine for this application is achieved through an anterolateral retroperitoneal approach. This approach provides excellent exposure of the entire lumbar spine with minimal muscle injury. In our experience, this approach diminishes postoperative pain and has lead to fast and reliable return to normal mobility and behavior. A 6-cm oblique skin incision is made between the twelfth rib and iliac crest, approximately halfway between the dorsal and ventral midline (Fig. 2, Left). A subcutaneous window is mobilized and the thin adventitial layer beneath the skin is swept using a dry gauze sponge to expose the underlying fascia and musculature. The junction between the thick white paraspinal fascia and the transverse running fibers of the external oblique muscle is identified (Fig. 2, Middle). The abdominal wall musculature is then incised in line with the skin incision approximately 2 cm

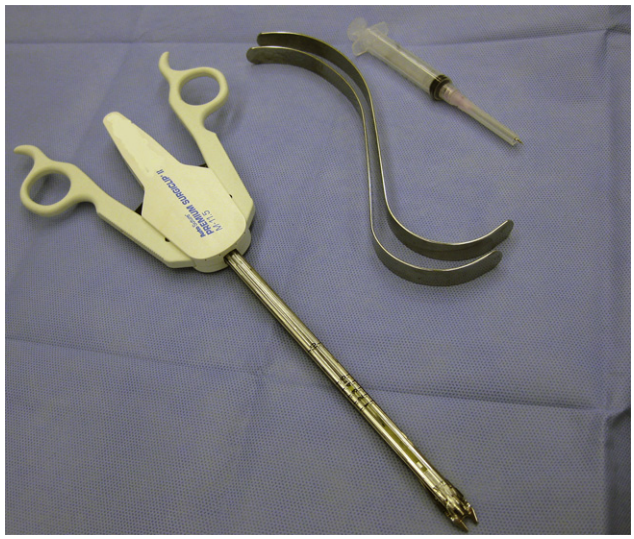

Fig. 1. Selected instruments, including a titanium vascular clip applier, double-ended handheld retractor, and 18G needle with 5-mm stopper on a syringe.