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Computed tomography-guided nucleus pulposus biopsy for canine intervertebral disc degeneration preparation: a radiology and histology study

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Abstract

BACKGROUND CONTEXT: Different animal models are being used in disc degenerative disease (DDD) research; most of these models are induced invasively rather than noninvasively. Computed tomography (CT)-guided percutaneous biopsy, widely used in clinical malignant pathology diagnosis, is a safe, useful, and highly accurate procedure. However, this process was not carried out in animal model preparation of intervertebral disc degeneration.

PURPOSE: To apply a minimally invasive method in DDD animal model preparation with a biopsy gun guided by CT scan and evaluate the accuracy and efficiency of this process with radiology and histology analyses. The relationship between the weight of removed nucleus pulposus (NP) and the degenerative process was also explored preliminarily.

METHODS: The canine intervertebral discs L1–L2, L3–L4, and L5–L6 were divided into three groups randomly: Group A: 18G biopsy gun; Group B: 20G biopsy gun; and Group C: 24G biopsy gun. After the lumbar spine was scanned with CT and the depth and angle of the centesis operation sites ascertained, biopsy gun was stabbed percutaneously through the annulus into the NP. A certain volume of NP tissue was removed by the biopsy gun. Radiology examination, including digital radiography (DR) and magnetic resonance imaging (MRI), was carried out preoperatively and at first and third months postoperatively. Each sample was harvested at the 3rd month postoperation for histology evaluation.

RESULTS: Computed tomography-guided percutaneous biopsy with 18G, 20G, and 24G biopsy guns was carried out for six discs, respectively. In the procedure, the weight of the NP tissue was removed: 3.0±0.53mg in Group A, 2.01±0.34 mg in Group B, and 0.99±0.12 mg in Group C. Significant differences of the weight of the removed NP tissue were calculated between groups ($p<.05$). During the observation period, although the disc height of Group A and B was reduced, no significant differences in the disc height comparison were found between groups at each time point and within groups at different time points in DR analysis. However, the signal intensity of MRI was reduced significantly ($p<.05$) in Group A when compared with Group C at third month, whereas the signal intensity was decrease mildly ($p>.05$) in Group B when compared with Group C at third month. The decreased content of NP, the number of NP cells, and the loose annulus fibrosus at the inner area was observed in both hematoxylin-eosin and Safranin-O staining in Group A at the 3rd month postoperation.

CONCLUSIONS: Computed tomography-guided percutaneous biopsy could be applied in intervertebral disc degeneration preparation of canine model, and the 20G biopsy gun would be the optimal choice in this procedure. The intervertebral disc degenerative process could be forecasted approximately on an account of the negative association between the degeneration process and the weight of the removed NP tissue.

Keywords: Animal model; Disc degeneration; Computed tomography; Minimal invasive surgery; Biopsy gun; Canine model

FDA device/drug status: Not applicable.

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Introduction

Low back pain is prevalent worldwide, which enhances the burden on social economy and decreases the quality of life of patients evidently [1–3]. Although various factors have been implicated in low back pain, disc degeneration is a significant risk factor for the development of such pain [4,5]. To study the underlying mechanisms of intervertebral disc degeneration and explore new therapeutic options, various disc degeneration animal models have been developed [6], among which the puncture injury model is used by many scientists and clinicians [7–9]. The aspiration effect, puncture depth, puncture times, and the gauge of needles are involved in affecting the process of disc degeneration in the puncture injury model [10,11]. However, nucleus pulposus (NP), lost in disc puncture procedure, is not considered as an important factor in the process of disc degeneration.

Nucleus pulposus, located at the center of the intervertebral disc, performs the duty of maintaining the balance between hydrostatic and osmotic pressures inside the disc. Integrity compromise of NP will lead to abnormal loading stress distribution and trigger fluid exudation and volume reduction. Subsequently, the disc undergoes a process of degeneration [12]. In this study, a minimal invasive, computed tomography (CT)-guided percutaneous NP biopsy procedure was conducted to manufacture disc degeneration in canine (Fig. 1). The relationship between the weight of the extracted NP and the degeneration process was discussed preliminarily.

Materials and methods

Source of animals

Animal experiments were performed in the Animal Laboratory, Navy General Hospital of PLA. Six 1-year-old female mongrel canines, weighing 10 to 12 Kg, were provided by the Animal Laboratory and placed in quarantine for at least 10 days before operation. Digital radiography (DR) (4mA, 68KV, GE, Fairfield, CT, USA) was performed to exclude animals with any abnormal structure (osteophyte formation, end plate calcification, Schmorl node, and the variation of lumbar vertebral body number) in the spine. T2-weighted sections of magnetic resonance imaging (MRI) (1.5 T, GE) of lumbar discs in the sagittal plane with spine coil were obtained (spin echo sequence with time to repetition of 2,500 milliseconds and time to echo of 85 milliseconds) and evaluated with Thompson classification [11] (1, normal; 2, minimal decrease of signal intensity but obvious narrowing of high signal area; 3, moderate decrease of signal intensity; and 4, severe decrease of signal intensity) to exclude animals with any lumbar disc degeneration sign (signal intensity reduction of NP, modic changes of end plate). The animal experiment protocols were approved by the local animal ethics committee (SYXK, June 2002–2011).

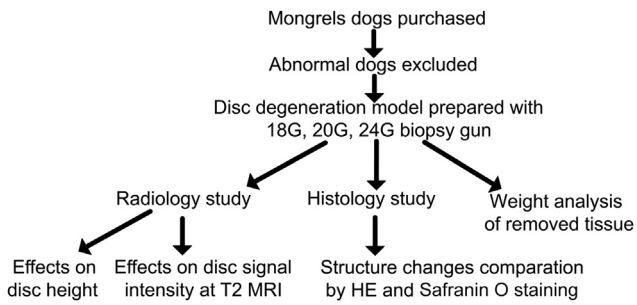

Fig. 1. The sketch diagram of intervertebral disc degeneration animal model preparation study. MRI, magnetic resonance imaging; HE, hematoxylin-eosin.

Disc degeneration model preparation

Computed tomography-guided percutaneous biopsy gun puncture combined with NP extraction technology was used to establish disc degeneration model in canine. In brief, the biopsy gun penetrated the center of the disc with CT guidance to obtain a certain amount of NP. The canine intervertebral discs L1–L2, L3–L4, and L5–L6 were divided into three groups randomly: Group A: 18G biopsy gun; Group B: 20G biopsy gun; and Group C: 24G biopsy gun.

General anesthesia with ketamine (10 mg/Kg) and midazolam (0.5 mg/Kg) was administered at the right forelimb of the canine. Skin preparation at the middle and left low back of the canine was conducted before operation. With right lateral recumbent position, the lumbar spine was scanned with CT (Brilliance iCT; Philips, Herrsching, Germany) in the following settings: scan time: 0.9 seconds, tube voltage: 140 kV, effective tube current: 300 mAs, and slice thickness: 3.0 mm. After puncture of operation sites, depth and angle were ascertained with a line marker (Fig. 2, Left), the skin of the operation sites were sterilized with iodophor. In left posterior route, the biopsy gun (COOK Medical Inc; Quick-Core, Bloomington, IN, USA) was punctured percutaneously through the annulus into NP (Fig. 2, Right) and confirmed with CT scan. The stylet of the biopsy gun was pushed out to grab NP tissues. The intervertebral discs in each group were operated respectively with this method. Blood loss, operation times, weights of removed NP tissues, and postoperative complications were registered. All animals were handled under the same condition. Magnetic resonance imaging and DR data were collected at the first and third months postoperatively. Intervertebral disc samples of each group were harvested at the third month for histology study.

Imageology study

The change of disc height was evaluated with three-center line method [10]. Briefly, on plain lateral film, four landmarks were chosen at the uppermost or lowermost corners