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Basic Science

Establishment of intervertebral disc degeneration model induced by ischemic sub-endplate in rat tail

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Abstract

BACKGROUND CONTEXT: Microcirculatory dysfunction of the sub-endplate is considered to reduce nutrient supply to the intervertebral disc (IVD); however, direct interruption or destruction of blood vessels in the bone marrow of the vertebrae body adjacent to the endplate has not yet been described, especially with regard to the calcification and ossification of the cartilaginous endplate occurring during IVD degeneration.

PURPOSE: The purpose of the study was to evaluate the causal relationship between IVD degeneration and blocking of the main blood supply gateway through the endplate.

STUDY DESIGN/SETTING: The study describes a new IVD degeneration model induced by ischemic sub-endplate.

PATIENT SAMPLE: A total of 40 Sprague-Dawley rats were included in the study group.

OUTCOME MEASURES: To assess disc height, a radiograph was taken each month for 4 months. Changes in endplate, nucleus pulposus (NP), and annulus fibrosus (AF) were evaluated by histochemical and immunohistochemical staining to detect IVD degeneration.

METHODS: Injection of 30 μL absolute ethanol into the IVD of rat tail at Co7/Co8 was used to induce injury. Controls were injected with 30 μL of phosphate-buffered saline into the IVD at Co8/Co9.

RESULTS: In the ethanol-injected group, disc height gradually decreased and bone sclerosis developed in the endplate. In the NP, cell transformation occurred, changing from predominantly vacuolar cells to chondrogenic cells and eventually fibrocartilaginous cells, along with fibrosis of the NP. As degeneration progressed, the AF developed disordered morphology and rough lamellae, and eventually ruptures and fibrosis. The extent of degeneration increased gradually over time, while the wavy tidemark of the growth plate regressed, and eventually disappeared. Initially positive collagen type II staining gradually decreased on the ischemic side of the sub-endplate. Except at the 3-month time point, expression of collagen type II, aggrecan, and Sox-9 in NP decreased gradually as degeneration progressed, compared with the control group.

CONCLUSIONS: This model successfully reproduced IVD degeneration, which could be used for etiological studies on IVD degeneration and investigation of nutrient supply disturbance, and may provide a theoretical foundation for clinical intervention and therapy for IVD degeneration in the future.

Keywords: Absolute ethanol; Endplate; Animal model; Intervertebral disc degeneration; Rat tail

FDA device/drug status: Not applicable.

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Introduction

The intervertebral disc (IVD) is the largest avascular tissue in humans. Most (80%) of the nutrient supply to the IVD is provided through endplate pathway diffusion, and it is also affected by the orientation of the outer layers of the annulus fibrosus (AF) [1]. Therefore, it is believed that a lack of nutrient supply from the blood vessels in the vertebral body could be involved in IVD degeneration. In addition, studies have shown that endplate degeneration is an initiating factor in IVD degeneration [2].

With aging, the endplate cartilage gradually becomes thinner, with development of calcification and even ossification, accompanied by reduction in the number of blood vessels. Consequently, there is much interest in identifying an antagonist against endplate cartilage degeneration to treat IVD degeneration. Research into repair and regeneration of degenerated IVD requires establishment of a suitable model to clarify the etiology and pathogenesis of disc degeneration, and thus explore the mechanism of lumbar degenerative disc disease, including its morphology, histology, cytology, and biochemistry. Animal models play an important role in imitating the human disc degeneration process. At present, in most of the established animal models, injury to the AF by needle puncture or blade stabbing is used to induce disc degeneration [3]. Although such experimental models have achieved a certain degree of success in mimicking the disc disease process, the changes in the AF through the process of disc degeneration occur uniformly throughout the disc structure, rather than being confined to the injury region [4]. Consequently, these models are not an accurate reflection of the pathophysiology and pathogenesis of the natural process of disc disorders or degeneration in humans, particularly with regard to the nutrient supply to the disc [5]. To reproduce the disturbance in blood supply, Turgut et al. [6] used subcutaneous injection of melatonin parallel to the endplates of injured vertebrae, which was able to reduce the cartilage endplate vascularity in degenerated IVDs by virtue of the osteoinductive role of melatonin in bone formation. In addition, nicotine inhalation through cigarette smoking resulted in disc degeneration by destruction of vascular buds in the vicinity of the vertebral endplate [7]. These previous reports indicate a relationship between disc degeneration and an impeded endplate nutrient supply; however, direct interruption or destruction of blood vessels in the bone marrow of the vertebrae body adjacent to the endplate has not yet been described, especially with regard to the calcification and ossification of the cartilaginous endplate occurring during IVD degeneration.

Ethanol as a method of protein denaturation is widely used in sclerotherapy for vascular malformations, because it causes injury to endothelial cells, with consequent vessel wall denudation and thrombus formation [8,9].

In this study, we explored a new method of destroying microcirculation in the vertebra through injection of absolute (100%) ethanol into sub-endplate of the vertebral body to assess the causal relationship between disc degeneration and blocking of the main blood supply gateway through the endplate. We hypothesized that this technique would first induce endplate degeneration, and that this would in turn lead to IVD degeneration, including degeneration of the AF and of the nucleus pulposus (NP). We chose to use the rat as our experimental animal, as injection into the rat tail is a simple operation, which is easy to reproduce and importantly, allows convenient observation and analysis of results.

Materials and methods

Study approval

Based on the protocol of 2011-0018, all experimental procedures were approved by Fudan University Chancellor's Animal Research Committee. This study was carried out in strict accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health.

Experimental animals

We used 40 skeletally mature male Sprague-Dawley rats in total (3 months old, body weight 356±9 g [mean±SD]) in our experiment. Thirty-two rats were treated with absolute ethanol and phosphate-buffered saline (PBS) injection respectively at the different sub-endplate level in individual caudal spine. Eight rats were used as the normal control group without any treatment to the normal IVD tissue. Experimental rats were euthanized; eight rats at random 1-month interval until 4 months after the injection.

Surgical procedure

Rats were anesthetized with an intraperitoneal injection of 5% ketamine hydrochloride (40 mg/kg) plus 0.5% diazepam (2 mg/kg). After induction of anesthesia, the surgical area of the tail skin was prepared by washing three times with povidone-iodine, and then draped with sterile drapes. Under X-ray fluoroscopy guidance, the vertebral body of Co8 at a point 3 mm from the junction of the Co7/Co8 discs was injected with 30 μL absolute ethanol into bone marrow of the vertebrae body using a 1-mL syringe attached to a 7G lumbar puncture needle, which was kept in position for 1 minute to achieve adequate diffusion of the alcohol, then removed. The surgical procedure was performed by a percutaneous, minimally invasive procedure (Fig. 1). Similarly, in the injection control group, Co9 was injected close to the Co8–Co9 disc junction with 30 μL PBS as described previously. Finally, the surface skin was re-sterilized. After the rats recovered from the anesthesia, they were housed in separate cages, and allowed to eat, drink, and bear weight ad libitum.