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Experimental intervertebral disc degeneration models

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Abstract

Intervertebral disc degeneration is a major health problem of close concern to both young and old. The problem is also growing as the global population ages. Intervertebral disc degeneration is defined as progressive changes affecting the spine as a component of natural aging under the effect of multiple factors (such as smoking, obesity, and incorrect exercise). For a solution to be found, experimental disc degeneration must first be induced, the causes of the disease must be identified, and early diagnostic and therapeutic methods must then be developed. Methods of inducing intervertebral disc degeneration with high applicability in rats were identified from the previous literature. This review discusses four methods of disc degeneration induction. It also discusses how to detect degeneration formation and development times. As a result of the literature review, information about four different and reliable intervertebral disc degeneration methods is presented.

Keywords: intervertebral disc, degeneration, animal models, rat

1. Introduction

Intervertebral disc degeneration (IDD) is a disease that may be seen at any age and may not be fully treatable. Cheung et al. (1) examined the distribution of IDD by age using magnetic resonance imaging and detected the condition in 40% of individuals aged under 30, and in more than 90% of those aged 50-55. This shows that IDD can be seen at any age, but that the incidence increases with age. However, some clinicians have reported no association between IDD and age, and describe the condition as having other pathological causes. There is, therefore, no current consensus on whether IDD develops due to age or pathological causes (1).

The elderly population is increasing worldwide. This is also leading to an increase in age-related health problems. In addition to old age (2), IDD can also emerge as a result of environmental factors (such as smoking, obesity, and incorrect exercise) (3). IDD is one of the principal causes of low back pain (4). More than 80% of adults approached health institutions due to low back pain at some time in their lives. Back pain is also one of the most common causes of restricted activity among individuals aged under 45 in particular (3). Disc degenerations are extremely painful, especially those seen in the early period (5). Back pain also affects the adult population, in addition to low back pain (6).

IDD caused low back pain has become an important problem due to increased costs and loss of working time for diagnosis and treatment (4). One study from the USA reported total low back pain-associated health costs of $91 billion. The health costs of individuals with low back pain exceed those without low back pain by approximately 60% (7). In addition, the incidence of IDD-related spinal surgery in the USA increased by 500% between 1990 and 2011 (8).

It is extremely difficult to develop and apply models for examining the pathogenesis of disc degeneration and assessing potential treatments (9). Disc degeneration is difficult to detect in humans and is usually identified after pain has occurred. Detection is particularly impeded by the slow progression of degeneration, the lack of disc degeneration tissue, and multi-factor underlying causes. Animal experiments are, therefore, fundamental in this context. The anatomical and biomechanical properties of the discs of both large and small animals are relatively similar to those of humans (10, 11). The purpose of this review is to summarize viable animal models of IDD.

2. Models of experimental intervertebral disc degeneration in animals

2.1. Animals Used

Both Sprague Dawley and Wistar rats have been used in these experiments (12-15). All animals were anesthetized during the surgical procedures.

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2.2. Methods Used

2.2.1. Intervertebral disc degeneration model induced with ovariectomy

Wang et al. (16) discovered during an experiment that rats subjected to ovariectomy also developed IDD. This method was subsequently developed based on that finding. Female Sprague Dawley rats were used for the model, and IDD has been reported to occur 12 weeks after surgical intervention (13).

Surgical procedure

Experiments begin when rats are three months old. The ovaries are removed (dual ovariectomy), and the incision area is sutured. Rats are sacrificed after 12 weeks of survival (13).

2.2.2. Intervertebral disc degeneration model created in the rat tail

This method is performed using the tail region of Sprague Dawley rats. IDD has been reported two weeks after the procedure Fernández-Susavila et al. (15).

Surgical procedure

During the procedure, a tourniquet is attached to the tail to reduce blood circulation in that region and the blood supply of the area to be studied.

Once the tail skin has been sterilized, a dorsal 4-cm midline incision is made between the C6 and C10 caudal vertebrates identified by digital palpation in the tail. A 1-mm diameter hole is created under the vertebral endplates using a precision drill. To control the depth of drill penetration, attention is paid to the resistance of the contralateral wall of the vertebral bone. The bone cavity is filled with cyanoacrylate to barrier any revascularization to the endplate, and the incision site is closed. The animals are sacrificed after two weeks (15).

2.2.3. Intervertebral disc degeneration model created by needle puncture

This method is applied to the coccygeal intervertebral region of Wistar rats weighing 300-350 g. IDD takes place 1-4 weeks after the procedure.

Surgical procedure

The coccygeal intervertebral spaces Co6-7, Co7-8, and Co8-9 are identified by digital palpation and confirmed by fluoroscopy. The intervertebral Co7-8 level is left untouched for control purposes, and no procedure is performed. The tail skin is first cleaned with antiseptic. A fluoroscopy-guided 20-gauge needle is then inserted at the Co8-9 (distal) and Co6-7 (proximal) annulus fibrosus level. After penetration, the needle is rotated 3600 and held in place for 30 seconds. The depth of penetration of the needle is controlled. For this, the resistance of the contralateral annulus fibrosus is checked during the procedure. After the procedure, the area is closed, and the animals are sacrificed after four weeks (12).

2.2.4. Intervertebral disc regeneration model created by Dedifferentiated Fat (DFAT) cell transplantation

Sprague-Dawley male rats (12 weeks old, bodyweight 300 g) were used in this model. IDD occurs in the eighth week after the procedure. Intervertebral disc degeneration is created as a result of a two-stage application.

Dedifferentiated Fat cell preparation

A new preadipocyte cell line, named dedifferentiated fat (DFAT), is created with mature adipocytes from adult rats (17). DFAT cells are easily isolated from about 1 g of subcutaneous adipose tissue and can be increased easily (14). 1g adipose tissue is taken under the skin of the rats. The adipose tissue is gently shaken at 37 ° C for 1 hour and kept in 0.1% (W / V) collagenase solution (Collagenase type 1, Koken, Tokyo, Japan). After filtration and centrifugation at 135g for three minutes, the floating top layer containing unilocular adipocytes is collected. Afterwards, the collected cells are washed with phosphate-buffered saline. Then, the cells (5x104) are placed in 25 cm2 culture flasks filled with Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 20% fetal bovine serum (FBS; JRH Bioscience, Lenexa, KS, Lot 6G2146). It is then incubated at 37 °C in 5% CO2. Cells float on medium and are waited until they stick to the upper inner ceiling surface of the bottle. After 7 days, the flasks are inverted, with the media removed and the cells at the bottom. The media are changed every 4 days until the cells merge. When cells divide occurs, the cells become available (14, 18). Using these methods, DFAT cells are obtained. These cells are then transferred to the intervertebral discs by surgical operation.

Dedifferentiated Fat cell transplantation

In rats, disc damage is created by the needle model (12). One week after intervertebral disc damage is done, DFAT cells (5 x104/50 ml PBS, DFAT group, n ¼ 13) are transferred to the damaged area. After the incision area is closed, animals are sacrificed eight weeks later (14).

2.3. Detection of intervertebral disc degeneration

Methods such as histology evaluations (Van Gieson, collagen VI, collagen fiber orientation using picrosirius red staining, hematoxylin-eosin, and immunohistochemistry), magnetic resonance imaging, micro-computed tomography, polarized light microscopy, light microscopy can be used to determine whether IDD is occurring (12-15, 19)

3. Result

IDD is still an unsolved problem for society. Scientists resort to all possible methods to solve this problem. Since there are restrictions on applications to the human body, the most appropriate approach is experiments on animals. In this review, we tried to bring together animal models of IDD.

Conflict of interest

There is no conflict of interest including any financial, personal

or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence this work.

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Authors' contributions

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