EVALUATION OF HYPERALGESIA AND HISTOLOGICAL CHANGES OF DORSAL ROOT GANGLION INDUCED BY NUCLEUS PULPOSUS

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ABSTRACT

Objective: To evaluate the hyperalgesia and histological abnormalities induced by contact between the dorsal root ganglion and the nucleus pulposus. Methods: Twenty Wistar rats were used, divided into two experimental groups. In one of the groups, a fragment of autologous nucleus pulposus was removed from the sacrococcygeal region and deposited on the L5 dorsal root ganglia. In the other group (control), a fragment of adipose tissue was deposited on the L5 dorsal root ganglia. Mechanical and thermal hyperalgesia was evaluated on the third day and the first, third, fifth and seventh weeks after the operation. A L5 dorsal root ganglion was removed in the first, third, fifth and seventh weeks after the operation for histological study using HE staining and histochemical study using specific labeling for iNOS. Results: Higher intensity of mechanical and thermal hyperalgesia was observed in the group of animals in which the nucleus pulposus was placed in contact with the dorsal root ganglion. In this group, the histological study showed abnormalities of the dorsal root ganglion tissue, characterized by an inflammatory process and axonal degeneration. The histopathological abnormalities of the dorsal root ganglion tissue presented increasing intensity with increasing length of observation, and there was a correlation with maintenance of the hyperalgesia observed in the behavioral assessment. Immunohistochemistry using specific labeling for iNOS in the group of animals in which the nucleus pulposus was placed in contact with the dorsal root ganglion showed higher expression of this enzyme in the nuclei of the inflammatory cells (glial cells) surrounding the neurons. Conclusion: Contact between the nucleus pulposus and the dorsal root ganglion induced mechanical and thermal hyperalgesia and caused histological abnormalities in the dorsal root ganglion components. These abnormalities were characterized by an inflammatory and degenerative process in the structures of the dorsal root ganglion, and they presented increasing intensity with longer periods of observation.

Keywords – Spine; Intervertebral Disk; Low Back Pain; Hyperalgesia; Intervertebral Disk Displacement; Wistar Rats

INTRODUCTION

The symptoms caused by disk herniation are related to mechanical compression of the lumbar nerve roots[1-3] and the inflammatory process provoked by the components of the nucleus pulposus. Nerve root compression occurs through extravasation of the content of the damaged intervertebral disk into the vertebral canal, which is a rigid and limited space. The inflammatory process

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Declaramos inexistência de conflito de interesses neste artigo

relating to lumbar disk herniation is caused by contact between the biochemical components of the nucleus pulposus and the nerve tissue[4-7]. The nucleus pulposus is a gelatinous structure present inside the intervertebral disk and surrounded by the fibrous ring. It is practically acellular and non-vascularized; it does not have lymphatic vessels and is composed of proteoglycans and water. Since it does not have any contact with the systemic circulation after embryogenesis, it is recognized as a foreign body when exposed to the immune system, thus triggering an inflammatory response characterized by an infiltrate of neutrophils, macrophages and T and B cells, around the herniated intervertebral disk[2,8]. The presence of pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-α (TNFα) has also been demonstrated in patients with lumbar disk hernias, thus corroborating the studies that have shown that there is an inflammatory component in the physiopathology of disk herniation symptoms[9,10].

It has been reported that the nucleus pulposus has the capacity to cause abnormalities in the structure of dorsal root ganglia that are characterized by an inflammatory process and cell apoptosis[11,12]. The aim of this study was to experimentally evaluate mechanical and thermal hyperalgesia and histological abnormalities in the dorsal root ganglia induced by contact with the nucleus pulposus.

MATERIAL AND METHODS

Twenty male Wistar rats weighing between 220 and 250 g were used, which were supplied by the Central Vivarium of the Ribeirão Preto School of Medicine, USP. Before carrying out the experiment, the animals were kept for two days in the local vivarium for acclimatization in cages measuring 40 x 60 x 20 cm, with a maximum of six animals per cage, under controlled temperature conditions (22 to 25°C) and dark/light cycling (12 x 12 h), with free access to water and food. The research project was approved by the local ethics committee for animal experimentation, and the study was conducted in accordance with the international ethical standards for the use of laboratory animals.

To carry out the surgical procedure, the animals were anesthetized by means of intraperitoneal injection (i.p.), with a 10% solution of ketamine (Ketamina Agener®), at a dose of 0.1 ml per 100 g of rat body weight, 2% xylazine (Anesedan®), at a dose of 0.07 ml per 100 g of body weight, and 5% fentanyl (Fentanest®) at a dose of 0.001 ml per 100 g of body weight. Before the operation, all the animals were administered a single dose of Veterinary Pentabiotic (benzathine benzylpenicillin) 600,000 UI, procaine benzylpenicillin 300,000 UI, potassium benzylpenicillin 300,000 UI, dihydrostreptomycin sulfate 250 mg and streptomycin sulfate 250 mg (Small Size Veterinary Pentabiotic®) intramuscularly, at a dose of 0.1 ml per 100 g of rat body weight. For the procedure of dorsal root ganglion removal for the histological study, intraperitoneal anesthesia was used, consisting of a solution of 4% chloral hydrate, at a dose of 1 ml per 100 g of body weight.

The experimental model for disk hernia was used in accordance with the method described by Grava *et al*[13], which consisted of removing a fragment of the nucleus pulposus from the sacrococcygeal region and then placing it in contact with the dorsal root ganglion of the fifth lumbar root (Figure 1). Removal of the nucleus pulposus material from the intervertebral disk was done in the sacrococcygeal region (base of the rat's tail) by means of an incision in the midline of the transition region between the fourth sacral vertebra and the first coccygeal vertebra. The intervertebral disc was exposed bilaterally and the nucleus pulposus material was removed by means of a transverse incision above the fibrous ring. This gelatinous material was collected, weighed on a precision balance (average weight of 4 to 5 mg) and then deposited at the location determined for this study (dura mater, dorsal root ganglion or nerve root). After concluding the procedure, the surgical wound was sutured as a single layer including the muscle fascia and the skin. The surgery to place nucleus pulposus material on nerve tissues was carried out with the aid of an optical lens with ten times magnification of the observation field. The structures (dura mater, dorsal root ganglion or nerve root) were exposed for the nucleus pulposus material to be deposited, by means of right-side partial hemilateral laminectomy and removal of the transverse vertebral process at the level established.

In the control-group animals, the same procedure was performed to remove the nucleus pulposus from the sacrococcygeal region and expose the L5 dorsal root ganglion, but a fragment of adipose tissue was placed on the ganglion.

Two experimental groups with 10 animals in each group were formed. Both groups were subjected to surgery to remove the fragment nucleus pulposus from the