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Walking analysis of rats subjected to experimental disc herniation

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Abstract In an attempt to evaluate whether experimental disc herniation can result in changes in walking pattern, presumably indicating pain, four groups of rats – sham ($n = 5$), disc puncture (NP, $n = 5$), displacement of nerve root and ganglion (DIS, $n = 5$), and the combination of disc puncture and displacement (NP + DIS, $n = 6$) – were assessed when walking in a Plexiglas corridor at day 2–14 after surgery. All surgical procedures were performed at the L4–5 level on the left side. Step length analysis showed that rats in the NP and DIS series had no difference between the legs initially, but a tendency towards slightly shorter left steps at day 8–12, whereas the animals in the NP + DIS series had slightly shorter right steps day 2–10. However, no statistically significant differences compared with the sham series could be detected for any of the groups. Nerve dysfunction on the operated side was only observed on one occasion in two rats and on 2

days in one rat, all from the NP + DIS series. Apparent limping was seen in three of the animals in the NP + DIS series and in one in the DIS series. Limping and nerve dysfunction only co-occurred on one occasion out of a total of 13 observations of limping, suggesting that the limping was induced by pain and not neurologic deficit. In conclusion, the combination of epidural nucleus pulposus and displacement induced a limping whereas nucleus pulposus or displacement alone did not. Assessment of limping thus seemed to provide adequate information of presence of pain, but step length measurement did not provide any useful data. Although walking analysis may be a valuable assessment of sciatic pain, better modalities must be developed to analyze experimentally induced nerve root pain.

Key words Sciatica · Intervertebral disc · Herniated disc · Spinal nerve roots · Nerve compression

Introduction

During recent years there has been an increasing number of experimental studies assessing the pathophysiologic mechanisms behind sciatica. These studies have shown that nucleus pulposus is not, as previously considered, a biologically passive substance that merely compresses the adjacent nerve root mechanically at disc herniation, but

instead a biologically highly active substance that may induce both structural and functional changes in the nerve roots [7, 8, 12–14]. There are also some studies that have tried to assess whether nucleus pulposus may induce pain when applied epidurally [7, 15]. These investigations have shown that application of either light mechanical deformation or nucleus pulposus on a nerve root does not induce any alterations in pain behavior assessed as changes in response thresholds to mechanical and thermal stimula-

tion, but if both nucleus pulposus and light mechanical compression are applied, there is a transient reduction in the threshold for heat stimulation, indicating hyperalgesia. However, these measurements are performed in the standing rat and changes in these thresholds reflect neuropathic pain. Sciatica is often increased by more movements of the affected limb and the spine. Although neuropathic pain may contribute to sciatica, we have reason to believe that sciatica may also be due to mechanical irritation of inflamed structures at motion [5, 6, 9, 17]. A better way to assess sciatica in an experimental setting might thus be to study rats exposed to experimental disc herniation at walking rather than at standing. The aim of this study was therefore to assess changes in relative step length between the two hind feet, presence of limping, and nerve dysfunction at walking in rats subjected to experimental disc herniation.

Material and methods

Twenty-one female Sprague-Dawley rats (bw 250 g) were anesthetized with an intraperitoneal injection 0.4 ml of diazepam:pentobarbital:saline (1:1:2). The thoracolumbar fascia was incised just left of the spinous processes of L4 and L5, and the erector spinae muscle was gently moved laterally to expose the facet joint between the two vertebrae. The left-side fourth lumbar dorsal root ganglion (DRG) the fifth lumbar nerve root and the L4–5 intervertebral disc could hereby be identified after removal of the L4–5 facet joint (Fig. 1A). The continued procedure was then dependent on the randomized test protocol.

Sham ($n = 5$). After exposing the nerve structures and disc the wound was closed.

Nucleus pulposus ($n = 5$). The L4–5 disc was punctured by a needle (0.4 mm diameter; Fig. 1B). After slow injection of air into the disc, with simultaneous manipulation of the lumbar spine, a herniation of nucleus pulposus occurred (Fig. 1C). Some of the herniated nucleus pulposus was transferred to the left fourth lumbar DRG.

Displacement ($n = 5$). A needle (0.4 mm diameter) was placed between the pedicle and the left fourth lumbar DRG. The needle was gently forced medially to the former location of the center of the DRG. At this position, the needle was drilled into the vertebral bone by rotating it between the fingertips. This induced a slight medial displacement of the left DRG and nerve root, and thus a light mechanical deformation.

Nucleus pulposus and displacement ($n = 6$). In this series, both disc puncture and displacement were performed.

The experimental protocol was approved by the local Animal Ethics committee.

Fig. 1 **A** View of the opened spinal canal after performing a facetectomy of the left L4–5 facet joint. The intervertebral L4–5 disc (1) is seen just caudal to the fourth lumbar dorsal root ganglion (2). The fifth lumbar nerve root (3) is seen medially to the exposed disc. The cut pedicles of the fourth (4) and fifth (5) lumbar vertebrae are also visible. **B** A needle with 0.4 mm diameter is introduced into the disc. **C** After inflating the disc with air, using a gentle pressure on the syringe, nucleus pulposus (*) is forced out into the spinal canal (bar represents 1 mm)

Fig. 2 Foot prints and walking patterns of the hind feet. To the *left* are normal foot prints and to the *right* are foot prints with nerve dysfunction indicated in the left foot. To the *left*, the toe spread (TS) and print length (PL) are indicated in the first foot print with the right foot, and judged to be normal. The first step with the right foot in the *right* walking track also demonstrates normal conditions. However, the steps with the left foot in the *right* walking track demonstrate a reduced toe spread and an elongated print length, indicating nerve dysfunction with muscular weakness in the left leg and foot. The measurements of step lengths are indicated by the horizontal lines. The step lengths in both the *left* and the *right* walking track demonstrate that the left and right steps are relatively similar. The rat producing the *right* walking pattern demonstrated a clear limping when walking, but this did not result in any apparent difference in step length between the left and the right side

Walking analysis

The rats were tested 1 day before surgery and then at day 2, 4, 6, 8, 10, 12 and 14 after surgery. The test comprised three different observations when the rats were allowed to walk three times in a Plexiglas corridor 13 cm wide and 49 cm long.

Step length

The hind feet were dipped in X-ray film developer (Kodak dental X-ray developer, Kodak Pathé, Paris) diluted with water (developer:water, 1:3). To make the solution more viscous, glycerin was added at a ratio of 1:3 to the diluted developer. Since the floor in the corridor was covered by a non-developed X-ray film (Fuji medical X-ray film, Fuji Photo Film Co., Tokyo), the foot prints of the hind feet, thus covered by the X-ray developer, were visualized on the film (Fig. 2). The average length of the steps performed with the right foot was divided by the average length of the steps of the left foot. A ratio higher than 100% thus reflected that the

steps taken with the right foot were on average longer than the left steps. The measurements were all performed blindly and after code breakage the step length ratios were organized according to experimental series and day of observation. Statistical differences between the series were assessed using Student's *t*-test, and $P < 0.05$ was considered to be statistically significant.

Nerve dysfunction

Nerve dysfunction was assessed on the foot prints obtained. Nerve dysfunction is seen as a characteristic narrowing of the width of the foot print and an elongation of the print length due to contact between the surface and the heel, which is usually not in contact with the ground (Fig. 2). The evaluation thus resulted in either a positive or negative judgment of nerve dysfunction for each rat and time point.

Limping

The rats were observed when walking and the presence of evident limping was noted. Limping was defined as present or not present as judged by the speed of walking, non-uniform motion pattern indicating unilateral discomfort in one of the hind limbs, and the impression that the rats were in pain when walking. The observer was not aware of the protocol for each rat and thus this evaluation was performed blindly.

Results

Step length

The processed data are presented in Fig. 3. There was a tendency for the animals of the nucleus pulposus (NP) and the displacement (DIS) series to have symmetric step lengths (close to 100%) and then slightly longer right steps (over 100%) at days 8–12. The animals in the combination series (NP + DIS) had values lower than 100%, indicating shorter steps with the right foot, which seemed to normalize within 10 days after surgery. However no statistically significant differences with respect to sham could be detected for any of the groups at any of the time points.

Nerve dysfunction

Nerve dysfunction was only detected in occasional animals in the combination series (Figs. 2, 4).

Limping

Apparent limping was observed in three of the animals in the combination series and a light limp at day 12 in one of the animals in the displacement series (Fig. 5). When a rat was limping, it moved slowly and was careful not to rest on the left foot more than necessary. The limping was pronounced in the three rats from the combination series,

Fig. 3 Average ratio between right and left step expressed as percentages, so that a ratio below 100% implies a shorter step with the right foot and values over 100% indicate a shorter step with the left foot. The values for the sham series are generally a bit lower than 100% but never less than 90%. The values for the disc puncture with leakage of nucleus pulposus (NP) series and the displacement of the fourth lumbar nerve root and dorsal root ganglion (DIS) series were initially close to 100% and later slightly higher during day 8–12, subsiding at day 14. The combination of NP and DIS induced a reduction of percentage step length indicating shorter steps with the right foot. This reduction was most pronounced at day 4 and was recovered at day 12. Although there was a clear tendency for the combination NP + DIS to result in shorter steps with the right foot, with no apparent changes for NP and DIS, there was no statistically significant difference compared with the sham series in any of the average step lengths

Fig. 4 Nerve dysfunction as assessed in Fig. 2 was only present on four occasions in three of the animals in the NP + DIS series. The observations from day 2 and 4 were from the same animal and the two other observations from two other animals

whereas the one occasion it was observed in the displacement series limping was more vague.

Limping and nerve dysfunction only co-incided on one occasion. When assessing the average step length of the 13 observations of limping animals, it was found to be

Fig. 5 Limping was observed on a total of 13 occasions. In the combination series (NP + DIS) there was one animal displaying limping from day 2 to day 14, one animal from day 8 to 14, and one animal only at day 10. The animal showing limping at day 4 also displayed nerve dysfunction

100% $\pm$ 29%. Average step length at the four observations of nerve dysfunction revealed two animals with shorter left steps and two animals with shorter right steps.

Discussion

The present study showed that rats exposed to an experimental disc puncture with herniation of nucleus pulposus or to slight mechanical nerve root deformation showed no apparent changes in step length and no nerve dysfunction or limping. However, if disc puncture and mechanical deformation were combined there was limping in three of six animals, and a tendency to have a shorter step with the right foot.

The biologic effects of nucleus pulposus have been evaluated in a large number of investigations during recent years. Nucleus pulposus is not an “inert” tissue that can compress nerve roots in cases of disc herniation, but has been found to be biologically highly active. Nucleus pulposus has been found to induce structural and functional changes in the adjacent nerve roots when placed epidurally [8, 12–14]. The role of nucleus pulposus in pain production has recently been evaluated [7, 15]. It seems that epidural presence of nucleus pulposus in combination with mechanical deformation may induce pain, whereas epidural application of nucleus pulposus per se does not when applied at a single level [15].

Walking track analysis has been found to be a valuable tool for assessing nerve dysfunction due to peripheral nerve injury [1–4, 10]. A special formula for calculating nerve dysfunction based on certain measurements of representative foot prints has been found to be both reproducible and of high sensitivity for detecting nerve dysfunction [1, 2, 4]. The calculation results in a Sciatic

Function Index (SFI) and reflects a reduction of the toe spread and an increase of the foot-print length due to the heel approaching the ground, which is also apparent when observing the foot-print. In the present study we use the technique of obtaining foot prints as previously presented [2]. However, our assessment did not include an SFI calculation, but a blinded assessment of the foot prints, thus only defining animals displaying characteristics of nerve dysfunction as described (Fig. 2). Therefore, this assessment did not result in a numeric value of sciatic function, but only a positive or negative judgment. For our purpose, however, this simple judgment of nerve dysfunction versus no dysfunction was considered adequate.

A blinded assessment of presence of limping was also included in the walking analysis of the present study. It was found that, except for one vaguely limping rat at day 12 in the displacement group, limping was only present in the series with both nucleus pulposus and displacement. Since nerve dysfunction and limping co-occurred only 1 of the 13 times limping was seen (Fig. 5), we suspect that limping was the result of pain rather than of nerve dysfunction.

Although limping in the human usually is combined with a restriction of step length, the present study failed to detect a clear difference in step length, possibly due to the high spread of the data. However, there was a tendency for disc puncture or displacement of the nerve root and ganglion alone not to induce any changes in step length, while the combination of nucleus pulposus and displacement did induce a reduction of the step length of the right foot. This could be interpreted as the animals being unwilling to stand on the left foot while moving the right foot. However, the average step length of the 13 observations of limping animals was $100\% \pm 29\%$, showing an equal step length for the two legs. Also, when looking at the step length at the four observations of nerve dysfunction, there were two animals with shorter left steps and two animals with shorter right steps. Based on these observations, we may conclude that step length assessment does not seem to be a valuable instrument for evaluation of nerve dysfunction, pain or limping. The most probable explanation for this result is that the step length at limp-

ing/pain may be compensated for in four-footed animals, and that it is therefore not as obvious as in bi-pedals.

Similar to previous observations, the present data indicate that neither exposure to nucleus pulposus nor mechanical deformation present as pain if applied at one level, whereas it may be observed if applied at three ipsilateral nerve root levels [7, 15]. However, the combination of epidural nucleus pulposus and slight mechanical deformation clearly produces limping and, as previously seen, a reduction in thresholds for thermal stimulation, when applied at only one level [15]. It is not clear, however, if this should be interpreted as a sensitization of the nerve tissue by nucleus pulposus to mechanical deformation or if this is simply the addition of two potentially painful events which both induce subclinical symptoms, respectively. From clinical observations it is known that touching an non-anesthetized nerve root exposed to nucleus pulposus can reproduce sciatica, and touching a nerve root not exposed to nucleus pulposus is not painful and merely produces slight discomfort [11, 16]. This could favor the hypothesis that there is, in fact, a sensitization of the nerve root by nucleus pulposus, but the exact pathophysiologic mechanisms can not be verified using the present experimental design.

In conclusion, walking analysis seems to be a valuable instrument for assessing sciatic pain only when considering presence of limping. However, techniques other than step length measurements must be developed, and such evaluation tools probably have to comprise lengthy recordings and analysis of motion patterns. The study also clearly indicated that the combination of epidural nucleus pulposus and mechanical deformation is necessary to induce limping that may be related to sciatic pain, which may thus support previous observations.

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