Effects of intervertebral disc lesion and multifidus muscle resection on the structure of the lumbar intervertebral discs and paraspinal musculature of the rat

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Abstract

The aim of this study was to investigate whether elimination of multifidus muscle in rats causes intervertebral disc (IVD) degeneration similar to that found after IVD lesion. Data were obtained from 36 male Wistar rats randomly assigned to one of three groups: (i) IVD lesion, in which the L4/L5 IVD was stabbed; (ii) multifidus muscle resection, in which all multifidus tissue between L3 and L6 was excised bilaterally; (iii) control, in which no intervention was applied. At 7, 14, and 28 days post-intervention, L4/L5 IVDs were harvested for histological analysis; left and right multifidus fascicles between L3 and S1 (from control and IVD lesion animals) and medial longissimus between L1 and S3 (from all animals) were dissected and weighed. ANOVA indicated significant group differences and a significant interaction between group and days for relative nucleus pulposus area and for multifidus mass normalized to body mass. No significant effects were observed for whole IVD area. At 14 days post-op, the IVD lesion group had a significantly smaller relative nucleus pulposus area than control and multifidus resection groups. Nucleus pulposus size did not differ from control at 7 and 28 days. At 7 days post-intervention, normalized multifidus mass was significantly lower (20%) in the IVD lesion group. For longissimus mass, no between-group differences were found. These results indicate that, in rats, IVD recovers quickly after lumbar IVD lesion and multifidus disruption does not cause IVD degeneration within the time studied.
Introduction

Low-back pain (LBP) is widely prevalent, is the largest cause of disability burden internationally (Hoy et al., 2014) and contributes substantial costs related to sick leave and medical consumption (Balague et al., 2012). Although LBP often recovers spontaneously, its recurrence is high (van den Hoogen et al., 1997; Von Korff, 1994), and it often develops into a chronic fluctuating problem with intermittent flares (Burton et al., 2004; Croft et al., 1998). The development of chronic problems accounts for the bulk of the costs associated with LBP (van Tulder et al., 1995; Von Korff, 1994). Unfortunately, the effect-size of current treatments for LBP is small. One explanation for this limited efficacy is that treatments are often applied without consideration that sub-groups of patients might require different treatments (Bouter et al., 1998), possibly because the mechanism for persistence of LBP remains elusive in most patients. For instance, a specific diagnosis is made in <10% of LBP cases (Deyo et al., 1992; Waddell, 1996).

One promising intervention for LBP includes rehabilitation of the function of the spinal muscles such as the lumbar multifidus (Hides et al., 2001). This approach is supported by the association between LBP and an impaired ability to recruit this muscle (MacDonald et al., 2009, 2010; Wallwork et al., 2009) and with localized atrophy of this muscle (Hides et al., 2008; Hides et al., 1996; Hides et al., 1994). However, it may be more effective for some individuals than others (Ferreira et al., 2007; Macedo et al., 2014; Vasseljen et al., 2012). A major limitation to progress in this field is the limited understanding of the mechanisms and effects of deficits in these muscles.

Rehabilitation of the multifidus muscles is based on the hypothesis that mechanical dysfunction of the spine contributes to LBP (Panjabi, 1992a; Panjabi, 1992b). It is thought that failure to maintain patterns of segmental motion under physiologic loads might underpin nociceptor discharge leading to pain. It is assumed that the multifidus plays an important role in stabilization of the spine, given its specific morphological properties (Ward et al., 2009a; Ward et al., 2009b) and its presumed ability to
provide segmental control in contrast to larger superficial muscles that span multiple motion segments (MacDonald et al.; Macintosh and Bogduk, 1986; Wilke et al., 1995).

A strong association between lumbar spine and multifidus muscle degeneration was recently observed (Shahidi et al., 2017). Causal links between IVD degeneration and multifidus muscle changes could be bi-directional. From one perspective IVD injury and/or degeneration may induce multifidus muscle changes. Human studies have identified atrophy within days after onset of back pain (Hides et al., 1994), but cannot exclude pre-existence of the changes. Animal studies have shown atrophy of multifidus muscle within days of experimental injuries (Hodges et al., 2006) and reduced excitability of spinal inputs to multifidus, but contrasting increase in corticospinal inputs (Hodges et al., 2009). Human data show no changes in multifidus muscle cross-sectional area at three months after onset of IVD herniation, but did reveal increased adipose tissue infiltration (Battie et al., 2012). Consistent with this observation are findings of extensive structural changes in muscle fiber types, connective tissues and fat in conjunction with IVD degeneration at six months after experimental injury in sheep (Hodges et al., 2015). Thus, IVD degeneration as a cause of multifidus change appears convincing, but the efficacy of multifidus muscle training for LBP is based on the opposite assumption, that compromised muscle changes have consequences for joint mechanics, and perhaps IVD degeneration.

Segmental mechanical dysfunction resulting from deficits in multifidus structure and activation could cause cumulative injury of annulus fibers, which have been shown to lead to IVD degeneration (Han et al., 2008; Issy et al., 2013; Kaapa et al., 1995; Kaapa et al., 1994; Osti et al., 1990; Rousseau et al., 2007). Although plausible, and critical for understanding the relationship between improved muscle structure and function of multifidus and LBP outcomes, it has not yet been established whether compromised multifidus muscle leads to any changes at the joint, which may be identified as more rapid development of IVD degeneration.

Unraveling the potential interplay between lumbar paravertebral muscles and IVDs requires an
animal model that allows manipulation and assessment of the state of the IVD and the multifidus muscle at multiple time-points. The aim of the present study was to use a rat model to investigate whether elimination of multifidus muscle causes IVD degeneration similar to that found after IVD lesion, and whether IVD lesion caused multifidus muscle atrophy. In the present paper, we provide evidence on effects of injury at a single lumbar IVD and of multifidus resection on the degeneration of the IVD and on the mass of the paravertebral musculature in the rat at 7, 14 and 28 days after the surgical interventions. We hypothesized that IVD injury would induce IVD degeneration and multifidus muscle atrophy, and that multifidus resection would induce IVD degeneration.

Methods

Data were obtained from 36 adult male Wistar rats (Rattus norvegicus) that were randomly assigned to one of three groups: (i) lumbar IVD lesion (n = 12), (ii) lumbar multifidus muscle resection (n = 12), (iii) control, no intervention (n = 12). Surgical and experimental procedures were in agreement with the guidelines and regulations concerning animal welfare and experimentation set forth by Dutch law, and were approved by the Committee on Ethics of Animal Experimentation at the ‘Vrije Universiteit Amsterdam’ (FBW 13-03).

Thirty minutes prior to the surgery, buprenorphine (Temgesic®, Schering-Plough, Maarssen, The Netherlands) was administered subcutaneously (dosage 0.1 ml/100 g body mass). The rats were deeply anesthetized using isoflurane gas (induction 3%, maintenance 1-2%). All surgeries were performed under aseptic conditions. Body temperature was monitored, and the anesthetic state was checked routinely by evaluating withdrawal reflexes. Directly after the surgery, one and two days post-surgery, carprofen (Rimadyl®, Pfizer animal health B.V., Capelle a/d Ijssel, The Netherlands) was administered subcutaneously (dosage 0.1 ml/100 g body mass). After recovery from the surgery, the rats (housed in pairs) could move freely in their cages with access to food and water ad libitum.
In the lumbar IVD lesion group, body mass at the time of surgery was 258 ± 7 g. The L4/L5 lumbar IVD was stabbed using a transperitoneal-ventral approach, similar to that described previously (Rousseau et al., 2004). With the rat in supine position, the peritoneal cavity was exposed by a longitudinal abdominal incision of the skin and linea alba of the peritoneum (5-6 cm). To obtain access to the ventral aspect of the L4/L5 IVD, the intestines were moved to the cranial side of the abdominal cavity, the aorta and vena cava were pulled to the left side, and the obliquus internus abdominis muscle was pushed laterally. The IVD was stabbed with a tenotomy knife fully penetrating the nucleus pulposus (depth 2.5 mm). The intestines put back in place, and peritoneum and skin were closed with running sutures (5-0 Vicryl, absorbable, Ethicon).

In the multifidus muscle resection group, body mass at the time of surgery was 267 ± 8 g. With the rat in prone position, the lumbar multifidus muscle was exposed by a longitudinal midline incision of the skin, and the superficial and deep thoracolumbar fascia from vertebrae L3 till L6. The fasciae were cut near the lateral borders of the spine. The lumbar multifidus consists of several bundles that originate from the articular and mammillary processes, run in a mediocranial direction, and insert onto the lateral aspect of the spinous processes, two to four vertebrae cranial to the vertebra of origin (Arnold, 2008; Brink and Pfaff, 1980). To negate the mechanical effects of fascicles spanning the L4/L5 IVD, all multifidus muscle tissue between L3 and L6 was excised bilaterally. The thoracolumbar fascia and the skin were closed with running sutures (5-0 Vicryl).

Lumbar IVDs (L3/L4, L4/L5, L5/L6), all left and right multifidus fascicles between L3 and S1 (from the control and IVD lesion animals), and left and right medial longissimus muscles between L1 and S3 (from all animals) (Brink and Pfaff, 1980) were harvested 7, 14, and 28 days post-intervention (n=4 for each group and time point). Rats were deeply anesthetized (intraperitoneally injected urethane) according to standard procedures in our laboratory (e.g., Maas et al., 2005) and weighed. After the muscles and IVDs were harvested, rats were euthanized with an overdose of intracardially injected...
pentobarbital sodium (Euthasol®, AST farma, Oudewater, The Netherlands) followed by double-sided pneumothorax. The lumbar spine was dissected free, the IVD were excised, frozen in liquid nitrogen and stored at -80°C until further analysis. The masses of the excised multifidus fascicles and the medial longissimus muscles were measured, averaged across sides, and normalized to body mass.

In a cryostat at -20°C, IVDs were sliced transversally in 12 µm sections. Five sections from the middle portion of the IVD were stained with Alcian Blue and Picrosirius Red (AB&PR). From photographs of these slices, the area of the whole IVD and the nucleus pulposus were assessed using ImageJ (http://imagej.nih.gov/ij/). In addition to nucleus and IVD area, the relative nucleus area was calculated as the ratio of nucleus to IVD area. Some of the IVDs were damaged, precluding analysis, which resulted in less than four data points for the following IVDs, groups and time points: n = 3 for L3/L4 in the IVD lesion and multifidus resection groups 7 and 28 days post-intervention, n = 3 for L5/L6 in the multifidus resection group 7 days post-intervention, n = 2 for L5/L6 in the control group 7 days post-intervention and in the IVD lesion group 14 days post-intervention.

As for small sample sizes normality tests can not be applied appropriately (Ghasemi and Zahediasl, 2012), we assumed that the sample was drawn from a normally distributed population. To test for differences in body mass at the time tissue harvesting and to assess effects of IVD lesion and multifidus resection on morphological parameters of the lumbar IVD (nucleus pulposus area, IVD area and their ratio), analyses of variance (ANOVA) were performed with intervention and time point as fixed factors. Similarly, to assess effects of IVD lesion and multifidus muscle resection on normalized muscle masses, ANOVAs were performed with intervention and time point as fixed factors. In case of a significant interaction, the effects of the intervention at each time point and those of time point for each intervention were tested using one-way ANOVAs in combination with Bonferroni corrected t-tests. Statistical tests were performed using SPSS (version 23, IBM, Armonk, NY, USA). Results were considered to be statistically significant at p<0.05.
Results

Body mass at the day of tissue harvesting was similar between groups and time points (Table 1). Only for the 7-days post-intervention time point, body mass of the control group was lower than that of the multifidus muscle resection group (p = 0.009).

Fourteen days after IVD lesion, absolute and relative nucleus area of L4/L5 IVDs were lower than in control animals (Fig. 1; for details on all statistical analyses see Supplementary materials). Values at 7 and 28 days were not different to control. A similar but smaller difference was found between the multifidus resection and control animals for area of the nucleus pulposus at 14 days (Fig. 1), but this was not significant for relative nucleus area (Fig. 1). The latter suggests that the absolute difference was driven by coincidentally larger IVDs in the control group. IVD area was not affected by either of the interventions. For the control group, significant differences in only the absolute nucleus area between time points (between 7 and 14 days and between 14 and 28 days) were found. Figure 2 shows typical examples of the images at 14 days after the interventions and a control sample.

In view of the small numbers of rats available at each time point, individual data of each group are presented in Figure 3. These data confirm that some changes in IVD morphology were present as early as 7 days after the IVD lesion in some animals, whereas at 28 days post-op, half of the IVDs appeared no different to the control animals. Although we do not have data at 14 days from these latter animals, we presume this indicates they have almost fully recovered after initial area loss. Interindividual variability was much smaller for the other two groups.

IVD morphology parameters, as described above, were assessed also for the neighboring IVDs (L3/L4 and L5/L6). Regarding the area of nucleus pulposus, effects were similar but differences between groups were substantially smaller than those found for L4/L5 (Fig. 1). Significant differences with the controls were observed only for the for IVD lesion group. The results of the control group at 14 days...
suggest that the larger areas were a characteristic of the whole spine. Significant effects of time point were found for absolute nucleus area of L5/L6 IVDs of the control (between 14 and 28 days) and multifidus resection groups (between 7 and 14 days). For the latter group, also a significant effect of time point was found for relative nucleus area of L5/L6 IVDs (between 7 and 14 days). For the neighboring IVDs, overall IVD area was not affected by either of the interventions.

For absolute multifidus mass, significant effects of intervention and time point were found (Fig. 4, top left). For normalized multifidus mass, group differences and a significant interaction between group and time point were found. At 7 days post-op, normalized multifidus mass was significantly lower (20%) in the IVD lesion group than control (Fig. 4, bottom left). No differences in multifidus mass were found between IVD and control groups at 14 and 28 days post-surgery. For the IVD lesion group, a significant effect of time point on normalized multifidus mass was found (between 7 and 28 days). For absolute and normalized longissimus mass, only significant main effects of time point were found (Fig. 4, right). Thus, for none of the time points between-group differences in longissimus mass were found.

Discussion

This study aimed to investigate whether elimination of the multifidus muscle causes IVD degeneration similar to that found after direct lesion to the IVD. As expected, we found a decreased absolute and relative nucleus area, evidence of IVD degeneration, and multifidus muscle atrophy following IVD lesion. However, in contrast with our hypothesis, we observed a reduced absolute but not relative nucleus area after multifidus muscle resection. Our objective was to induce mechanical dysfunction at the L4/L5 level by complete removal of the multifidus muscle’s contribution. Segmental stability was not directly tested and could have been compensated by stiffening of the segments by scar tissue formed in response to the surgery or compensation by activation of the remaining lumbar
muscles. However, we did not observe any stiff connective tissue along the segments from which the multifidus was resected and no significant hypertrophy of the retained longissimus muscle.

Failure to observe changes in the IVD following removal of the multifidus muscle may have several mechanisms. The effects of multifidus resection may have a different time course, possibly requiring longer than 28 days to manifest into changes of nucleus size. Because we found clear indications of nucleus recovery 28 days after IVD lesion, it appears unlikely that multifidus resection will decrease the size of the nucleus after that. Although rats are often observed to sit on their hind legs, they are quadrupeds and this may explain the observations. Multifidus muscle has been suggested to play a specific stabilizing role in humans (e.g., Wilke et al., 1995), but our results cannot confirm such a role. However, stability of the spine may be less problematic in quadrupeds, where the spine can possibly be considered to be a stable arch (Aspden, 1989). This could also explain the better potential for IVD injury to recover in the rat lumbar spine (as we also observed) compared to the tail (Osti et al., 1990; Rousseau et al., 2007).

The lack of impact of multifidus muscle resection on IVD morphology might also be explained by the apparent resilience of the rat IVD. Our findings regarding IVD lesion concur with previous studies using different animal models (Osti et al., 1990; Rousseau et al., 2007). As we based our surgical approach on that described by Rousseau et al. (2004; 2007) and we used the same animal model (adult rat), a close comparison of our data to theirs was deemed most relevant. After stabbing three in-series rat tail IVDs, Rousseau et al. found a decrease in relative nucleus area as early as 7 days post-intervention (from 50% in control samples to 20% of IVD area after lesion). No clear reduction in nucleus size was found 4 days post-intervention, a time point not included in the present study. After 14 and 28 days, the size of the nucleus was decreased (0-12.5%) in some animals, but was somewhat increased in others (25-33%). Substantial variation between animals was found also in the present study. Besides a reduced size of the nucleus, Rousseau et al. reported an irregular nucleus shape as well biochemical
changes, such as an increased proteoglycan expression. However, no changes in bending mechanics were observed. Other studies have reported that gross-morphological signs of IVD degeneration (e.g., reduced nucleus area), as used in the present study, correlate with biochemical changes such as reduced water content, increased collagen content and decreased cross-linking of collagen (Han et al., 2008; Issy et al., 2013; Kaapa et al., 1994). Sampling after stabbing three in-series IVDs of the lumbar spine (L3/L4, L4/L5 and L5/L6) was limited to 28 days post-intervention (Rousseau et al., 2007). At this time point, the size of the nucleus was similar to that of controls (approximately 33% of IVD area) and an irregular shape was found in only 6 of 11 IVDs. This was hypothesized to reflect a better capacity to recover for lumbar compared to tail IVDs. Our data, which measured the short-term effects of a single lumbar IVD lesion for the first time, indicate healing of the lumbar IVD within 28 days after lesion, but only in half of the rats tested. With the present data, we cannot confirm if all IVDs will eventually recover or if some IVDs remain disrupted. The indications of recovery of the IVD at day 28 may be specific for the rat. In sheep, IVD stab injury progresses to degeneration and, except for the peripheral annulus fibrosus, there is no indication of repair (Osti et al., 1990). In contrast to Rousseau et al. (2007), we stabbed a single IVD (L4/L5). Despite this local intervention, we found changes of nucleus size in the adjacent IVDs (L3/L4 and L5/L6). This indicates that adjacent segments cannot be used as controls. The nature of rat IVD to recover, even after direct lesion, which initiates the cascade of degeneration in other species, may suggest resilience of the IVD that limits the potential for muscle dysfunction to induce changes in the IVD.

Similar to previous results in pigs (Hodges et al., 2006), we found that IVD lesion induced rapid (as early as 7 days post-intervention) atrophy of the lumbar multifidus muscle. As multifidus resection did not cause clear IVD degeneration, our results imply that, at least for rats, the direction of causality in the association between lumbar IVD and multifidus muscle degeneration is from IVD to muscle. This may suggest a possible mechanism underlying the link between LBP and multifidus atrophy (Hides et al.,
We found no hypertrophy of longissimus muscle to compensate for multifidus atrophy. The apparent recovery of multifidus muscle mass concurs with the absence of reduced multifidus muscle cross sectional area at three months after IVD herniation in humans (Battie et al., 2012) and three and six months after IVD lesion in sheep (Hodges et al., 2015). Both of those studies identified that although muscle size was not changed, connective and/or adipose tissue content increased. It is unclear whether muscle composition was changed in our rat model of IVD lesion.

In this study, no sham surgery was performed as control. For the dorsal approach (multifidus muscle resection), a sham surgery would involve only cutting the skin as any further dissection will already affect the muscle. This was deemed not to have added value to the control group used here. Absence of a sham surgery for the ventral approach, may have confounded the IVD lesion results by damage of the ventral abdominal wall. Given the absence of effects of multifidus resection, substantial effects of the minor damage to the abdominal muscles, for example due a reduced level of physical activity (not monitored in the present study), seem unlikely. Each group in the present study included a relatively low sample number (n = 4; n < 4 for some L3/L4 and L5/L6 IVDs). This increases the influence of individual samples that are closer to the extremes of the Gaussian distribution, which most likely explains the higher absolute nucleus areas of the control group at 14 days. Substantial differences were identified with rather low p-values (see Supplementary materials) indicating sufficient power. However, for smaller differences, such as found at time points other than day 14, type II errors cannot be excluded. In addition, we used parametric statistics in our analysis in spite of the fact that the small sample does not allow verification of a normal distribution of the data. Given the nature of the variables studied, we assumed that the data can be considered as a sample from a normal distribution, but the statistical results should be interpreted with some caution.
We conclude that in the rat, lumbar IVD lesion results in IVD degeneration and multifidus atrophy, but unlike other species, the rat IVD appears to recover within 28 days after injury. On this foundation of IVD resilience, resection of the multifidus muscle does not appear to cause IVD degeneration. However, we cannot exclude the possibility that muscle resection might accelerate IVD degeneration or delay the recovery process. The effects of simultaneous IVD lesion and multifidus resections need to be studied in the future.

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Conflict of interest statement

We have no competing interests.

References


Legends to figures

Figure 1. Group data of morphological parameters for the L3/L4, L4/L5 and L5/L6 discs. Area nucleus pulposus (left), intervertebral disc (IVD) area (middle), relative nucleus area (i.e., nucleus area divided by disc area; right) for the three experimental groups at each of the three time points after intervention. The error bars denote one standard deviation; asterisks denote significant post-hoc differences between groups (p < 0.05).

Figure 2. Typical examples of sections of the L4/L5 intervertebral discs stained with Alcian Blue and Picosirius Red (see Methods) from each of the experimental groups at 14 days post-op and at matched body mass for the control group. IVD – intervertebral disc.

Figure 3. Individual data of the L4/L5 disc nucleus pulposus area for the three experimental groups at each of the three time points after intervention.

Figure 4. Group data of absolute (top) and relative (normalized to body mass, bottom) mass of multifidus and longissimus muscles for the three time points after surgery. As no regeneration of muscle tissue was observed following multifidus resection, multifidus mass is shown for the control and IVD lesion groups only. The error bars denote one standard deviation; asterisks denote significant post-hoc differences between groups (p < 0.05). MF, multifidus.
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<table>
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<th>Group</th>
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<th>28-days post-op</th>
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<td>IVD lesion</td>
<td>282 ± 9</td>
<td>300 ± 10</td>
<td>350 ± 11</td>
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<td>Multifidus resection</td>
<td>293 ± 13</td>
<td>316 ± 13</td>
<td>371 ± 7</td>
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<tr>
<td>Control</td>
<td>254 ± 18*</td>
<td>312 ± 11</td>
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N = 4 for each experimental group and time point. Asterisk denotes a value significantly different from the multifidus resection group (p = 0.009).