Effects of compressive loading on biomechanical properties of disc and peripheral tissue in a rat tail model

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Received: 29 September 2008 / Revised: 2 April 2009 / Accepted: 10 June 2009 / Published online: 26 June 2009
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Abstract Intervertebral disc degeneration induced by mechanical compression is an important issue in spinal disorder research. In this study, the biomechanical aspect of the rat tail model was investigated. An external loading device equipped with super-elastic TiNi springs was developed to apply a precise load to the rat tail. By using this device, rat tail discs were subjected to compressive stress of 0.5 or 1.0 MPa for 2 weeks. Discs in the sham group received an attachment of the device but no loading. After the experimental period, first the intact tail with peripheral tissues (PT) such as tendon and skin and then the retrieved disc without PT were subjected to a uniaxial tension–compression test; biomechanical characteristics such as range of motion (ROM), neutral zone (NZ), and hysteresis loss (HL) were evaluated. Furthermore, the load-bearing contribution of PT in the intact tail was estimated by comparing the load–displacement curves obtained by the mechanical tests performed with and without PT. The experimental findings revealed that the continuous compressive stress induced reduction in disc thickness. The

intact tail demonstrated decreases in ROM and NZ as well as increases in HL. On the other hand, the retrieved disc demonstrated increases in ROM, NZ, and HL. Further, a significant increase in the load-bearing contribution of PT was indicated. These findings suggest that the load-bearing capacity of the disc was seriously deteriorated by the application of compressive stress of 0.5 or 1.0 MPa for 2 weeks.

Keywords Intervertebral disc · Compression load · Biomechanical properties · Load-bearing capabilities · Rat tail model

Introduction

Many cases of low back pain are considered to be associated with intervertebral disc degeneration, of which excessive mechanical loading is regarded as one of the risk factors [9, 10, 29, 42]. To elucidate the mechanism by which such degeneration occurs and progresses, several studies have been performed, including in vitro experiments on cultured disc cells [11–13] and tissues [17, 18, 41] under mechanical stresses. The murine tail disc model has been frequently adopted in animal model studies for producing disc degeneration-like changes by the application of artificial mechanical loading because it is inexpensive and convenient to perform.

The murine tail model was first introduced by Lindblom [22, 23], in which the rat tail was fixed in a U shape, and cell death in the annulus fibrosus was demonstrated. Lotz et al. [26] used the mouse tail disc compressed by cantilever metal springs and revealed apoptosis, a decreased expression of Type II collagen in all regions of the disc, and a decreased expression of aggrecan proportional to

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the decreased cell density in the nucleus pulposus (NP). Similarly, Court et al. [7, 8] applied bending stresses to the mouse tail disc and showed increased cell death and decreased aggrecan gene expression on the concave side in the annulus fibrosus. Iatridis et al. [16] applied compression to the rat tail disc and showed an increased proteoglycan content in the disc. On the other hand, Hutton et al. [15] suspended rats with their tails to apply tensile force to the discs and showed a decreased proteoglycan content in the disc. Thus, this model had successfully enabled researchers to perform biological and biochemical analyses of the degenerated discs under mechanical loading.

On the other hand, the biomechanics of this tail model have not been fully investigated. For example, Lotz et al. [26] showed that a neutral zone in the mouse tail disc after continuous compression was significantly increased versus control. Iatridis et al. [16] reported that an angular laxity of the rat tail disc after continuous compression was significantly decreased versus control. Since the angular laxity measured by a bending test could be considered to be similar to the neutral zone measured by a uniaxial test, the studies seemingly yielded opposite results. This difference possibly originates from the difference in the animal models, loading methods, and mechanical testing methods; however, no detailed analysis of the cause of this controversy has yet been reported. Noticing the biomechanical investigations on human cadaveric discs, it was demonstrated that continuous compression reduces the volume of NP, which leads to a reduction in the disc stiffness and then to a deterioration in the load-bearing capacity of the disc [1, 2, 45]. Thus, the biomechanical characteristic of the tail disc is one of the key factors for understanding the disc degeneration process. It is therefore necessary to resolve these uncertainties.

Hence, in the present study, to address these problems, we performed a detailed biomechanical analysis of the rat tail model. We prepared a precise external loading device and carried out an animal experiment in which the tail disc was subjected to continuous compression for 2 weeks. Uniaxial tension–compression tests on the intact tail with peripheral tissues (PT) such as skin and tendons as well as the retrieved disc without PT were conducted, and the biomechanical characteristics of the tail and disc were

obtained. Then, the impact of the continuous compression on the load-bearing function of the disc was examined.

Materials and methods

Animal experiments

A total of eighteen 12-week-old male Sprague–Dawley (SD) rats were used in this study. The intervertebral disc between the fifth and sixth tail vertebrae was subjected to continuous static compression by using an external loading device described later. The animals were randomly assigned to one of three groups. The discs of rats in the 0.5-MPa group ($n = 6$) were subjected to static compression of 0.5 MPa for 2 weeks. Those in the 1.0-MPa group ($n = 6$) were similarly subjected to compression of 1.0 MPa. The discs in the sham group ($n = 6$) were shams; the devices and protective capsules were attached to the tails but no loading was applied. All animal experiments were conducted in accordance with the protocol approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University and in compliance with the committee's guidelines.

Loading devices

To apply a static compression to the rat tail disc, we prepared an external loading device as shown in Fig. 1. The device weighed 6 g and consisted of four stainless steel pins, two aluminum ring plates, aluminum bolts and nuts to fix the rings to the pins, and super-elastic springs made of TiNi alloy [Light (509-21) or Heavy (509-23) types; Tomy, Ohkuma, Japan] installed between the rings.

Force–elongation behavior of each spring was measured using an electromagnetic material testing machine (MMT-250N; Shimadzu, Kyoto, Japan) at room temperature. The springs exhibited super-elastic characteristics after the initial linear elastic deformation. In animal experiments, the springs with a natural length of 3.2 mm were installed on the device with an elongation of approximately 8 mm. At this elongation, the traction force of the light-type spring was 1.07 ± 0.02 N (mean $\pm$ SD, $n = 8$) and that of

Fig. 1 Loading device for rat tail disc. **a** Schematic drawing of the loading device. Eight super elastic springs installed between two plates generated a compressive load on the intervertebral disc. **b** Device attached to a rat tail by stainless steel pins inserted into the vertebrae

the heavy-type was 2.41 ± 0.02 N ($n = 8$). Eight light-type springs were used to generate the compression load of 8.56 N for the 0.5-MPa group, and the same number of heavy-type springs to generate a load of 19.28 N for the 1.0-MPa group. Since the cross-sectional area of the disc of a 12-week-old male SD rat was 17.69 ± 0.74 mm² ($n = 12$), these loads induced a compressive stress of 0.48 and 1.09 MPa in the discs of the respective groups.

Attachment of loading devices

On attaching the loading device to the rat tail, we maintained the animal in inhalation anesthesia with 2% isoflurane (IsoFlo; Abbott Laboratories, North Chicago, IL, USA) in oxygen. Two 0.8-mm diameter stainless steel pins (501-28; Tomy) were inserted percutaneously into the midsection of the fifth vertebra such that they were perpendicular to the longitudinal axis of the tail and crossed each other. In the same manner, two more pins were inserted into the midsection of the sixth vertebra. After the confirmation of correct pin positioning with radiographs, the aluminum ring plates were fixed to the pins using bolts and nuts. Further, an aluminum capsule weighing approximately 20 g was mounted for covering the device. On the next day (the second day of the experiment), the springs were installed between the two plates to apply compressive loads in the 0.5-MPa and 1.0-MPa groups, as shown in Fig. 1b. The animals were kept in cages without any constraint and fed ad libitum for 2 weeks of the experimental period.

X-ray examinations

X-ray images of the discs in the ventral–dorsal direction were taken on the first day, just before the application of compression, and on the 16th day, after the 2-week experimental period. The disc thickness was measured by the method proposed by Lay et al. [20] with slight modifications. Briefly, the X-ray photographs were scanned using a flatbed scanner at a resolution of 4,800 dpi and transferred to a computer. The image was analyzed using an image-processing tool (HALCON; MVTec, Munich, Germany), in which we placed a region of interest (ROI) with half the width of the proximal vertebra. After the determination of ROI borders between the vertebra and disc by edge analysis, the disc thickness was obtained as the average thickness of the ROI.

Mechanical tests

To investigate the biomechanical properties of the disc, we carried out an *in vivo* mechanical test (hereafter designated the wPT test) of the intact disc specimen with PT using the

electromagnetic testing machine (MMT-250N). We then performed an *ex vivo* mechanical test (hereafter designated the woPT test) after sacrifice and removal of PT. The discs in all the groups were evaluated by both wPT and woPT tests.

The wPT test was carried out on the 16th day of the experimental period. The animal was anesthetized, and its intact tail was attached to the testing machine using pins inserted in the tail. The test was performed in a load-controlled mode; the tail was subjected to cyclic axial loading of sinusoidal waveforms at a frequency of 0.1 Hz and in the range of 16 to -16 N. The first nine loading cycles were applied for preconditioning, and the load–displacement curve in the tenth loading cycle was adopted for the analysis. In a pilot study, it was confirmed that these loadings did not generate damages in specimens.

The woPT test was carried out following the wPT test and sacrifice of the animal via anesthetic overdose. The tail was resected and PTs around the disc were removed. The vertebrae–disc–vertebrae specimen thus prepared was mounted on a testing machine, and a similar mechanical test was conducted. The strength between the vertebrae and the endplate was very weak, leading to the failure of the specimens under a tensile force of 16 N; hence, the peak tensile load was determined to be 5 N as described later. Thus, the cyclic load applied was of a sinusoidal waveform at a frequency of 0.1 Hz and in the range of 5 to -16 N. The data were analyzed for biomechanical characteristics in the similar manner as in the wPT test.

Biomechanical characteristics

Biomechanical characteristics of the disc, such as range of motion (ROM), neutral zone (NZ), and hysteresis loss (HL), were calculated from the measured load–displacement curve, as illustrated in Fig. 2. The characteristics evaluated by the wPT and woPT tests will hereafter be referred to as ROM/wPT and ROM/woPT, respectively.

First, the average load–displacement curves for wPT and woPT tests were calculated as the relationship of the mean load at loading and unloading versus the displacement. ROM/wPT and ROM/woPT were determined as the range of deformation accompanying the respective load range. NZ/wPT and NZ/woPT were obtained as the range of deformation in the average load–displacement curve corresponding to the load ranging from 2 to -2 N, in which 2 N is approximately half of the animal's body weight [26]. HL/wPT and HL/woPT were defined as the area enclosed by the loading and unloading parts of the load–displacement curves [5, 34].

The PT around the disc as well as the disc itself served as the load-bearing members of the tail. The loads before and after the removal of PT were obtained using the wPT

Fig. 2 Load-displacement curves and biomechanical characteristics. **a** Typical load-displacement curve of the disc in the sham group measured with the peripheral tissue (wPT test). Range of motion (ROM) was defined as the peak-to-peak displacement difference under the axial load ranging from -16 to 16 N. Neutral zone (NZ) was the range of average displacement corresponding to the load ranging from -2 to 2 N. Hysteresis loss (HL) was defined as the area enclosed by the loading and unloading parts of the load-displacement curves. **b** Typical load-displacement curve of the disc in the sham group measured after the removal of PT (woPT test). ROM was defined as the peak-to-peak displacement difference under axial load ranging from -16 to 5 N. Definition of NZ, and HL were same as in the wPT test

and woPT tests, and the difference between the loads yielded the load borne by PT. After matching the origins of both curves, the magnitudes of displacement were obtained

at a load of 1 , -1 , -8 , and -16 N on the average load-displacement curves obtained by wPT; then, the differences of the load at these displacements were calculated and the load-bearing ratios were obtained. The loads of 1 and -1 N were in the range of NZ, and those of -8 and -16 N comprised the continuous compression load applied in the experiments.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to detect the effects of the experimental conditions (sham, 0.5 MPa compression, and 1.0 MPa compression) on the weight of the rats, the disc thickness, and the biomechanical characteristics of ROM, NZ, and HL. Two-way ANOVA was performed to detect the effects of the experimental conditions and experimental period on the disc thickness. When significant effects were detected, post hoc multiple comparisons were performed using the t test with significance levels adjusted by the Bonferroni–Holm correction. Statistical differences were assumed for $P < 0.05$. All statistical analyses were carried out using an open-software R (available at <http://www.r-project.org/>).

Results

Animal condition

In all animal experiments, no serious complications, including infection, were observed. No damage in the loading devices or loosening of the pins occurred. The attached devices and capsules caused no obstruction in the rat's movement; apparently, they did not suffer any obvious discomfort. The animal weighed 395 ± 23 g (all groups, $n = 18$) at the beginning of the experiment, 446 ± 17 g (sham group, $n = 6$), 457 ± 24 g (0.5 -MPa group, $n = 6$), and 470 ± 22 g (1.0 -MPa group, $n = 6$) at the end of the experimental period. One-way ANOVA followed by post hoc multiple comparisons demonstrated that the weight increased significantly in all groups ($P < 0.01$), but no significant differences were observed between the groups ($P > 0.05$).

Disc thickness

The X-ray photographs demonstrated that no bending deformations were induced between the adjacent vertebrae. The disc thicknesses measured by image analysis are shown in Fig. 3. Two-way ANOVA demonstrated that the experimental period and conditions had a significant effect on the disc thickness ($P < 0.01$). Post hoc multiple comparisons showed significantly lesser disc thickness in the

Fig. 3 Disc thicknesses after the experimental period of 2 weeks. Data are presented as mean $\pm$ SD. Asterisks indicate a significant difference with $P < 0.01$ compared to the sham group; parallel lines indicates a significant difference between loading groups with $P < 0.01$. The disc thicknesses of compression groups were significantly lesser than that of shams ($P < 0.01$). Further, the thickness of the 1.0-MPa group was lesser than that of 0.5-MPa group ($P < 0.01$)

compression groups than in the sham group ($P < 0.01$), and significant differences between the two compression groups ($P < 0.01$). Although the post hoc t test showed that disc thickness decreased significantly over time in all groups ($P < 0.01$), the reduction ratio versus the initial thickness was considerable as 41 and 23%, respectively, in the compression groups as compared to 84% in shams.

Mechanical characteristics of the disc

Average load–displacement curves obtained are shown in Fig. 4. The curves expressed a nonlinear S-shaped relationship accompanied with large NZ and HL. In the wPT test, the compression groups demonstrated stiffer behaviors than the sham group. Contrarily, in the woPT test, the compression groups demonstrated more compliant behaviors than the sham group. Mechanical characteristics of ROM, NZ, and HL were derived from the average load–displacement curves and are presented in Fig. 5. One-way ANOVA showed the significant effect of the experimental conditions on the mechanical characteristics of the intact tail and the disc, and post hoc multiple comparisons indicated the significant differences as described below.

ROM/wPT decreased to 92% (0.5-MPa) and 73% (1.0-MPa) versus that in the sham group ($P < 0.01$), and NZ/wPT decreased to 65% (0.5-MPa) and 36% (1.0-MPa) versus that in the sham group ($P < 0.01$). Contrastively, ROM/woPT increased to 137% (0.5-MPa) and 153% (1.0-MPa) versus that in the sham group ($P < 0.01$), and NZ/woPT increased to 146% (0.5-MPa) and 157% (1.0-MPa) vs. that in the sham group ($P < 0.01$). HL/wPTs increased to 144% (0.5-MPa) and 165% (1.0-MPa) versus that in the

Fig. 4 Typical load–displacement curves of the discs in sham and compression groups after the 2-week experimental period. Data are presented as mean $\pm$ SD. The curves expressed a nonlinear S-shaped relation. In the case where samples were tested with the peripheral tissues (wPT test), the compression groups demonstrated stiffer behaviors than did the sham group. In contrast, after the removal of the peripheral tissues (woPT test), the compression groups demonstrated more compliant behaviors than the sham group

sham group ($P < 0.01$), and HL/woPTs increased to 179% (0.5-MPa) and 264% (1.0-MPa) versus that in the sham group ($P < 0.05$ and $P < 0.01$, respectively).

Load borne by peripheral tissues and load-bearing ratio

The load borne by PT and the load-bearing ratio are presented in Fig. 6. No significant differences between the groups were found under the loads of 1 and -1 N, while a significant difference was detected at -8 N between the sham and 1.0-MPa groups ($P < 0.05$). Further, the compression groups demonstrated significantly greater values than sham group at -16 N ($P < 0.01$ or $P < 0.05$). The load-bearing ratio of the PT in the 1.0 MPa group was very large and reached up to 94%.

Discussion

Rationale of the experiment

An animal model using murine tails to investigate the disc degeneration induced by mechanical compression is referred to as a tail model, and this model has been used in several studies. In these studies, static compressive loads were applied to the discs via pins inserted into the adjacent vertebrae. The compressive load was created by coiled metal springs [14, 16], cantilever metal springs [26], or

Fig. 5 The range of motion (ROM), neutral zone (NZ), and hysteresis loss (HL) of the disc obtained by the test with the peripheral tissues (wPT test) and the test without the peripheral tissues (woPT test). Data are presented as mean $\pm$ SD. *Double asterisks* indicate a significant difference with $P < 0.01$ and *asterisk* with $P < 0.05$ compared to sham group; *parallel lines* indicate a significant difference between the compression groups with $P < 0.01$ and *continuous line* with $P < 0.05$. NZ/wPTs in the compression groups were lower than that in the sham group, while NZ/woPTs in compression groups were greater than that in the sham group

Fig. 6 Load borne by peripheral tissues (PT) and load-bearing ratio. Data are presented as mean $\pm$ SD. *Double asterisks* indicate a significant difference with the sham group with $P < 0.01$ and *asterisk* with $P < 0.05$. No significant differences between the groups were found under the load of 1 N and -1 N, while a significantly greater load was borne by PT in the 1.0-MPa group than sham at -8 N ($P < 0.05$). Further, the compression groups demonstrated a significantly greater load borne by PT than sham at -16 N ($P < 0.01$ or $P < 0.05$). The load-bearing ratio in the 1.0-MPa group was very large and reached up to 94%

elastic rubber bands [25]. These springs and bands effectively generated the required loads; however, there was some concern with regard to their use. In the case of rubber bands, the load would decrease considerably during the experimental period due to the degradation of the materials. In the case of metal springs, the loads would decrease in accordance with the compression of the discs. Hence, in this study, we prepared a compact and lightweight loading device equipped with super-elastic springs, which enabled us to maintain the compressive load virtually constant even though the disc was compressed during the experiments. X-ray examination revealed that the disc thickness was reduced by 0.658 mm in the 0.5-MPa group and by 0.827 mm in the 1.0-MPa group, whereas the variation in the compressive stress induced by the springs was kept as low as 0.48–0.43 and 1.09–0.95 MPa, respectively. Further, since the total weight of 26 g corresponded to the load of approximately 0.25 N, which was less than 3% of the compression of the 8.56-N load applied to the 0.5-MPa compression group; the effect of weight of the device and the capsule on the tissue was considered to be small.

The magnitude and period of loading in this experiment were determined on the basis of the results of previous

studies using the tail model [25], which reported that disc degenerations were induced under continuous compressive stresses of 1 MPa applied for 7–14 days. This magnitude of stress was observed in human lumbar discs in daily activity [32, 37, 43, 44]. Hence, the compressive stress of 0.5 and 1.0 MPa in our experiment was within the physiological level, although the loading period was longer than the physiological one [30].

Several mechanical tests have been used to evaluate the biomechanical properties of the disc. Iatridis et al. [16] performed an *in vivo* axial dead-weight compression test of the disc with PT (wPT test), and Lotz et al. [26] performed a four-point bending test of the retrieved vertebra–disc–vertebra specimen without PT (woPT test). These tests have their own advantages for evaluating the disc characteristics; however, from the wPT test alone, we cannot separately obtain the biomechanical characteristics of the disc. On the other hand, the woPT test under a bending load provided us the response of the disc against bending load; however, in that case, we would encounter a difficulty in distinguishing the responses against the tensile load versus a compressive load since the bending load generates tensile stress in the extension side of the disc and compressive stress in the flexion side. Considering that the volume reduction of the NP causes a malfunction of the disc in bearing the compressive load [33, 39], it was important to determine the mechanical behaviors of the discs under both the tensile and the compressive loads to examine their load-bearing capabilities. Furthermore, the compression test, in which the disc did not experience a tensile load, did not provide a proper evaluation of ROM and NZ. Hence, in this paper, we performed a uniaxial tension–compression test of the disc specimen with and without the peripheral tissues.

With regard to the peak tensile load in the biomechanical test, it is known that the strength between the vertebrae and endplate is so weak that separation occurs during the course of degeneration experiments [4, 28, 31]. In our pilot studies, in which, a biomechanical test of the disc was performed with various loading conditions, we also experienced such separation under a tensile load of 6 N. Hence, in this study, we determined the peak tensile load to be 5 N and carried out the biomechanical test accordingly.

ROM, NZ, and HL were measured as the biomechanical characteristics of the disc. For the definition of NZ, we adopted the range of deformation corresponding to the load ranging from 2 to −2 N. The measured load–displacement curves, however, demonstrated nonlinear responses as shown in Fig. 4, in which NZ/wPT ranged over the linear response regions and NZ/woPT remained within the toe regions. Thus, NZ/woPT possibly gave us the values related to the lesser loading on tissues than those related to NZ/wPT. Hence, we did not perform a comparison between NZ/wPT and NZ/woPT but within the respective test groups.

Reduction in disc thickness

X-ray measurement revealed that the disc thicknesses decreased in proportion with the increase in the compression among the groups, as shown in Fig. 3. The reduction in thickness was also observed in the shams, similar to a previous report [6]; however, the cause was considered to be immobilization [16] but not compression. The reduction in disc thickness by compression has also been reported in previous reports [6, 16, 21], and is considered as the result of fluid loss under a compressive load. According to the studies of rehydration of the disc using cadaveric specimens [38], the disc thickness was recovered after immersion in a saline solution, and hence, the change was not persistent but reversible. However, a recent study using a rat model [21] reported that the disc thickness following compression of 11 or 17 N for 2 weeks was not recovered after an *in vivo* recovery period of 20 days. Furthermore, cell death in NP following mechanical compression has been confirmed in similar studies [25, 26]. Taking these reports into consideration, the change in disc thickness is considered to be persistent or at least to continue over the range of several weeks. There still remains the possibility that the recovery will take place after a very long period, and further investigation is necessary to elucidate disc remodeling during and after the compressive loading.

Impact of peripheral tissues on the biomechanical properties of the disc

With regard to ROM and NZ, the values obtained in the wPT tests demonstrated significantly lower values in the compression groups than those in the sham group. In contrast, those obtained by woPT tests showed significantly greater values in the compression groups than those in the sham group. Iatridis et al. [16] reported that the angular laxity of the disc measured by the wPT test was significantly lesser than that in the controls. Lotz et al. [26] showed that NZ of the disc measured by the woPT test was significantly greater than that in controls. Our results in which the biomechanical characteristics were evaluated by both wPT and woPT tests using the same specimens were consistent with these results reported separately and were a clear demonstration of the difference between wPT and woPT tests.

This notable difference between the results of the wPT and woPT tests indicated the considerable increase in the stiffness of peripheral tissues in the compression groups. Chronic arthrodesia induces an articular contracture through the increases in crosslinking in collagen [36] and the decrease in water content [3]. A decrease in ROM after 2-week immobilization in rat knee joints has been reported

[40]. Hence, we considered that the stiffening and contracture in the peripheral tissues were induced by the restriction of movement accompanied with the attachment of the loading device and the compression of the disc.

Impacts of the disc degeneration on the biomechanical properties of the disc

The disc thicknesses decreased in proportion with the increase in the compression; although a significant difference was shown between the two compression groups, the difference between them was lesser than that between the compression groups and sham group. Similarly, ROM/woPTs and NZ/woPTs in the compression groups were significantly greater than those in the sham group although the difference between the compression groups was lesser than those between the compression groups and sham group. These results supported the finding [19, 35] that NZ/woPT and ROM/woPT increased in accordance with the decrease in water content in NP under compression. More specifically, our experiments demonstrated that continuous compression of 0.5 MPa, which generated a comparable reduction in disc thickness as did the compression of 1.0 MPa, also induced comparable increases in NZ/WoPT and ROM/WoPT to those found under the compression of 1.0 MPa. These were a reflection of the malfunction of the load-bearing mechanism of the disc induced by the volume loss in NP. Actually, the failure of the load-bearing mechanism of the disc was apparent in the results concerning the load borne by the peripheral tissues, as shown in Fig. 6. The load-bearing ratio under 16 N in the sham group was approximately 50%; however, those in the compression groups were as large as 90%. These results suggested that the contracted peripheral tissues were the main load-bearing members in the tail, and at the same time, that the load-bearing capacity of the disc was seriously deteriorated.

Limitations

Although this study was performed to elucidate the disc degeneration process, it has several limitations stemming from the differences between the rat tail disc and human disc. For example, Lotz pointed out that the rat tail disc is more flexible than the human disc [27]. The differences in disc sizes and cell types have also been mentioned [24]. Further, the degeneration-like changes in these animal models comprise an acute response, whereas the degeneration observed in clinical cases is a result of chronic response, including tissue remodeling. These factors should be considered while analyzing and comparing disc disorders in humans and the results obtained from animal model.

Acknowledgments This study was partially supported by a Grant-in-Aid for Scientific Research (B) No. 17300145 and (B) No. 20300153 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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