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ORIGINAL ARTICLE

Comparison of the invasiveness of conventional discectomy and microendoscopic discectomy for lumbar disc herniation: Differences in the methods of approach

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Abstract

Introduction: The aim of this study was to investigate whether differences in the methods of approach to the vertebral arch influence the invasiveness of conventional discectomy and microendoscopic discectomy (MED).
Methods: In this study, 41 Wistar rats were divided into four groups: controls (no surgery) ($n = 10$), shams (skin incision only) ($n = 11$), MED ($n = 10$), and conventional discectomy ($n = 10$). We performed ethological and blood biochemical examinations for three of the groups, excluding the control group, and a histological examination for three of the groups, excluding the sham group. In the ethological examination, we measured the threshold of postoperative pain using the von Frey test. In the blood chemical examination, we measured blood creatine phosphokinase and inflammatory cytokines, and compared the severity of tissue damage by histological examination using hematoxylin–eosin and immunohistochemical staining.
Results: The conventional discectomy group showed a significantly lower threshold of postoperative pain, compared with the MED group ($P < 0.05$). Blood biochemical investigation revealed that the creatine phosphokinase ($P < 0.05$) and tumor necrosis factor-α levels ($P < 0.05$) of the conventional discectomy group were significantly higher than those in the MED group. In the histological examination, it was found that a wide range of paraspinal muscle damage occurred in the conventional discectomy group ($P < 0.05$) and that the damage was mostly confined to the periosteum and nearby nerve endings.
Conclusion: MED was found to be less invasive than conventional discectomy based on ethological, blood biochemical, and histological examinations.

Introduction

Since it was first reported by Foley and Smith in 1997 as a less invasive surgical procedure for lumbar disc herniation, microendoscopic discectomy (MED) has become common in Japan (1–3). Compared with MED, conventional discectomy leads to worse postoperative pain, because it requires separation of a larger range of the periosteum from the bone during the approach, nerve endings in a wider range of the periosteum are damaged, and more of the paraspinal muscles (primarily the multifidus muscles) are damaged. In contrast, MED is considered to lead to less postoperative pain and to be minimally invasive, because it does not require the separation of the periosteum from the spinous process and vertebral arch and the use of a tubular retractor is less invasive to the paraspinal muscles.

To date, various examinations have been conducted (4–6), including evaluation of inflammatory responses through postoperative blood sampling and pain evaluation using a visual analog scale (7–9). However, these evaluations have focused on the overall operative procedures, including disc removal operations, and to our knowledge, there has been no objective evaluation of the

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differences in invasiveness between these approaches to the vertebral arch.

Therefore, we examined the levels of invasiveness of different approaches to the vertebral arch in MED and conventional discectomy in rats using ethological, biochemical, and histological end-points.

Materials and Methods

This study was approved by the Ethics Committee of Toho University (approval number: 09-11-93).

We used 41 6-week-old male Wistar rats (body weight range, 165–190 g). The rats underwent general anesthesia by intraperitoneal administration of 0.001-mL/g pentobarbital (concentration, 64.8 mg/mL).

In order to develop MED in rats in the same manner as in humans, we designed a dilator and a tubular retractor for rats. The sizes of the dilator and the tubular retractor were determined based on the body length ratio between humans and rats. The dilator has a diameter of 3 mm and a length of 60 mm, and the tubular retractor has an inner diameter of 3 mm and a length of 40 mm (Tanaka Medical Instruments, Tokyo, Japan) (Figure 1).

Figure 1 Surgical instruments. Our original dilator (upper part). Tubular retractor (lower part).

Rats were randomly divided into the following four groups: controls, shams, MED, and conventional discectomy. In order to clarify the pain threshold values in rats, inflammation was induced at the surgical wound site with the injection of carrageenan (10).

Control group (n = 10)

Because normal tissues were to be examined in this group, neither anesthesia nor surgery was performed, and carrageenan was not administered.

Sham group (n = 11)

Rats received an approximately 2-cm longitudinal incision in the skin of the lower back at the center of the spinous process of the fourth lumbar vertebra. About 1 hour after incision, 100-μL carrageenan was injected into the wound, and the wound was sutured except for about 5 mm in the central part (Figure 2a).

MED group (n = 10)

Rats received a skin incision in the same manner as the sham group, and an additional small incision was made in the fascia of the left paraspinal muscle for insertion of

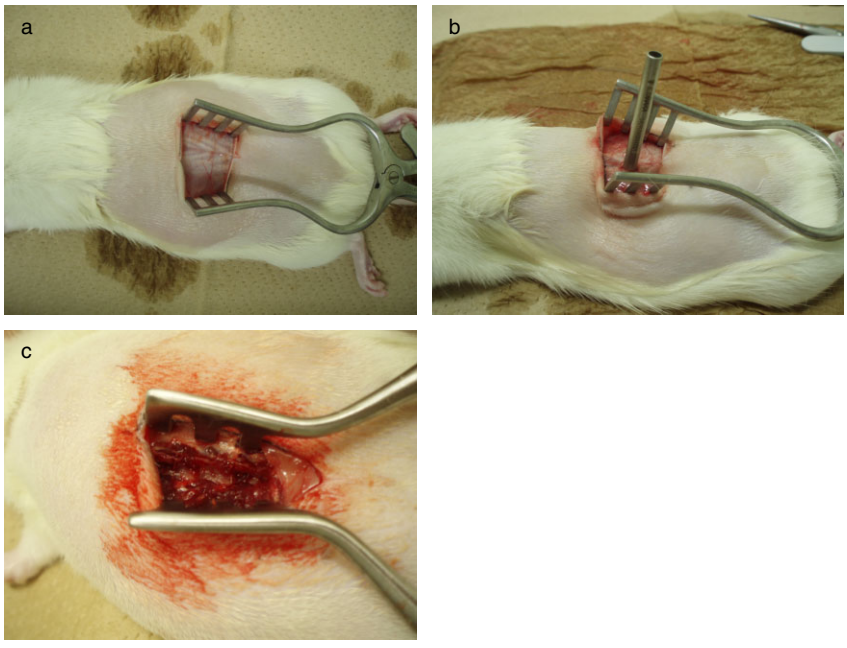

Figure 2 Operation methods. (a) Sham group: Rats received an approximately 2-cm skin incision, and the wound was sutured except for about 5 mm in the central part. (b) MED group: Rats received a skin incision in the same manner as the sham group, but also underwent a small incision in the fascia of the left paraspinal muscle through which a dilator was inserted. When the dilator reached the vertebral arch, a tubular retractor was inserted. After the tubular retractor had been placed for about 1 hour, the tubular retractor was removed, the fascia was sutured, and the skin was sutured in the same manner as the sham group. (c) Conventional discectomy group: The paraspinal muscles were separated from the spinous process and the vertebral arch with a raspatory beneath the periosteum, and the vertebral arch was exposed. The wound was then retracted for about 1 hour after injury. The fascia and the skin were then sutured in the same manner as sham group. MED, microendoscopic discectomy.

a dilator. When the dilator reached the vertebral arch, a tubular retractor was inserted. The tubular retractor was placed for about 1 hour, after which 100-μL carrageenan was injected into the wound, the tubular retractor was removed, the fascia was sutured, and the skin was sutured in the same manner as the sham group (Figure 2b).

Conventional discectomy group (n = 10)

After the rats received a skin incision in the same manner as the sham group, the paraspinal muscles were separated from the spinous process and the vertebral arch using an elevator with a raspatory beneath the periosteum, and the vertebral arch was exposed. The wound was then retracted with a Jansen retractor. About 1 hour after wounding, 100-μL carrageenan was injected into the wound, the Jansen retractor was removed, the fascia was sutured, and the skin was sutured in the same manner as sham group (Figure 2c).

Procedures

Ethological and blood biochemical examinations were performed on 11 rats in the sham group, 10 rats in the MED group, and 10 rats in the conventional discectomy group. Histological examinations were performed on 10 rats in the control group, 10 rats in the MED group, and 10 rats in the conventional discectomy group.

In order to correctly compare the invasiveness of approaching the vertebral arch in the sham, MED, and conventional discectomy groups, the development from the skin to the fascia was standardized. All rats in the three groups recovered spontaneously from general anesthesia within about 30 min.

Twenty-four hours after the operation, 100-μL carrageenan was again injected into the wound of each rat in the sham, MED, and conventional discectomy groups, and the von Frey test was applied 2 hours later to assess the threshold values of postoperative pain (11,12). In this measurement, two evaluators independently stimulated the fascia through the wound. When the rat exhibited escape behavior, the behavior was considered to be positive, and the mean of the positive target forces between the two evaluators was determined.

After completion of the von Frey test, blood was sampled from the inferior vena cava under inhalation anesthesia with diethyl ether, and the vertebral body was removed en bloc with the paraspinal muscle.

In the control, MED, and conventional disectomy groups, the vertebral body was removed en bloc with the paraspinal muscle under inhalation anesthesia with diethyl ether. After formalin fixation, the resected samples were sectioned for hematoxylin–eosin (HE) staining and immunostaining with anti-growth associated protein-43 (GAP-43) polyclonal antibody (#AB5220, lot #603024647; Chemicon International, Inc. Billerica, USA) and goat anti-interleukin-6 (IL-6) antibody (#Sc-1265, lot #G3008; Santa Cruz Biotechnology Inc., Santa Cruz, USA).

In IL-6 staining of the paraspinal muscles, the area of sites in which IL-6-producing cells were observed and the numbers of IL-6-producing cells per unit area were determined with a VANOX microscope (Olympus, Tokyo, Japan) and the DP71 operating system (Olympus).

Serum was obtained by centrifuging the blood sample (1500 *g* for 15 min), and C-reactive protein (CRP), creatine phosphokinase (CPK), IL-6, and tumor necrosis factor-α (TNF-α) levels were measured.

A touch-test sensory evaluator (Muromachi Kikai, Tokyo, Japan) was used as the hair for the von Frey test. The touch-test sensory evaluator has 20 different sizes, and each target force has been previously established, as shown in Table 1.

Table 1 Touch-test sensory evaluator

Evaluator size	Target force (g)	Evaluator size	Target force (g)
1.65	0.008	4.56	4.0
2.38	0.02	4.74	6.0
2.44	0.04	4.93	8.0
2.83	0.07	5.07	10.0
3.22	0.16	5.18	15.0
3.61	0.4	5.46	26.0
3.84	0.6	5.88	60.0
4.08	1.0	6.10	100
4.17	1.4	6.45	180
4.31	2.0	6.65	300

The evaluator has 20 different sizes, and the target force is set for each of them.

In the blood biochemical examination, CRP levels were measured with a nephelometer, CPK levels were measured according to the Japan Society of Clinical Chemistry reference method, and IL-6 levels (IL-6 Rat ELISA Kit: #KRC0061, lot #549972A; Invitrogen, Carlsbad, USA) and TNF-α levels (TNF-α Ultrasensitive Rat ELISA Kit: #KRC3014, lot #549972A; Invitrogen) were measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions.

Tukey's multiple comparison test was used to statistically assess differences from the ethological and blood biochemical examinations. The Mann–Whitney U-test was used to examine differences based upon the histological examination. $P < 0.05$ was considered to be significantly different.

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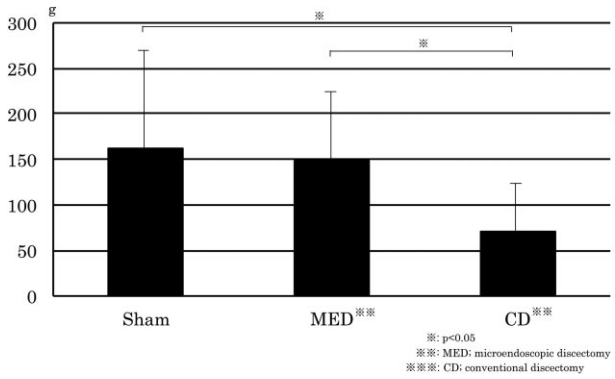

Figure 3 von Frey test. Threshold values of postoperative pain by the von Frey test showed that there was no significant difference between the sham and MED groups, but that the conventional discectomy group had significantly lower threshold values than the sham and MED groups. CD, conventional disectomy; MED, microendoscopic discectomy.

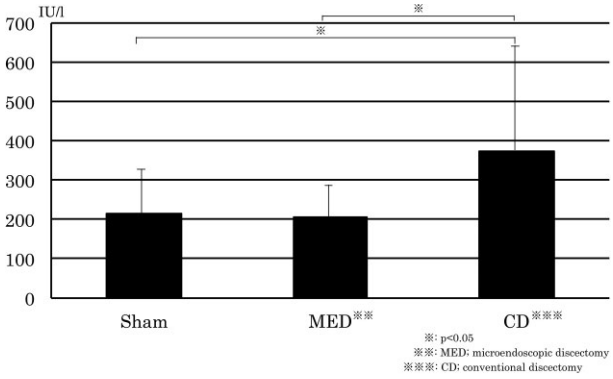

Figure 4 Serum CPK level. CPK levels were significantly different between the sham and conventional discectomy groups and between the MED and conventional discectomy groups. CD, conventional disectomy; CPK, creatine phosphokinase; MED, microendoscopic discectomy.

Results

Ethological examination

The threshold values of postoperative pain by the von Frey test were 60.0–300.0 g (mean, 161.8 g) in the sham group, 26.0–300.0 g (mean, 148.6 g) in the MED group, and 10.0–100.0 g (mean, 71.1 g) in the conventional discectomy group. There was no significant difference between the sham and MED groups, but the conventional discectomy group exhibited significantly lower threshold values than the sham and MED groups (Figure 3).

Blood biochemical examination

CRP levels were below the detection limit for the assay in all three groups.

CPK levels were 142.0–416.0 IU/L (mean, 216.4 IU/L) in the sham group, 129.0–351.0 IU/L (mean, 204.9 IU/L) in the MED group, and 119.0–770.0 IU/L (mean, 375.6 IU/L) in the conventional discectomy group. Significant differences were found between the sham and conventional discectomy groups, as well as between the MED and conventional discectomy groups (Figure 4).

Serum IL-6 levels were below the detection limit in the sham group, but were 62.60–429.00 pg/mL (mean, 220.94 pg/mL) in the MED group and 82.20–413.00 pg/mL (mean, 177.59 pg/mL) in the conventional discectomy group. There was no significant difference between the MED group and the conventional discectomy group (Figure 5).

Serum TNF-α levels were 2.30–4.60 pg/mL (mean, 3.39 pg/mL) in the sham group, 2.30–5.10 pg/mL (mean, 3.39 pg/mL) in the MED group, and 2.70–14.10 pg/mL (mean, 6.22 pg/mL) in the conventional discectomy group. There was no significant difference between the sham and MED groups, but the levels in the conventional discectomy group were significantly higher than the sham and MED groups (Figure 6).

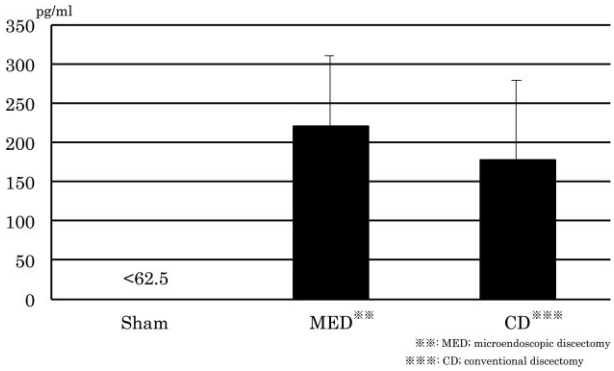

Figure 5 Serum IL-6 levels. Serum IL-6 levels were below the limit of detection in the sham group. There was no significant difference between the MED and conventional discectomy groups. CD, conventional disectomy; IL-6, interleukin-6; MED, microendoscopic discectomy.

Histological examination

HE staining

In the MED group, degeneration of the paraspinal muscles and inflammatory cell infiltration were observed along the site where the tubular retractor was inserted. Moreover, the periosteum disappeared at the sites where the tubular retractor was in contact with the vertebral arch (Figure 7a).

In the conventional discectomy group, a wide range of muscle degeneration was evident, and the periosteum disappeared completely at the sites at which the

paraspinal muscles were separated from the spinous process to the vertebral arch (Figure 8a).

Immunohistochemical staining

GAP-43 staining confirmed the presence of nerve endings in the periosteum in the control group. In the MED group, the presence of nerve terminals was confirmed at the sites at which the residual periosteum was confirmed by HE staining (Figure 7b,c). In the conventional discectomy group, the presence of nerve endings on the bone surface was not confirmed because the periosteum disappeared completely at the developed sites (Figure 8b).

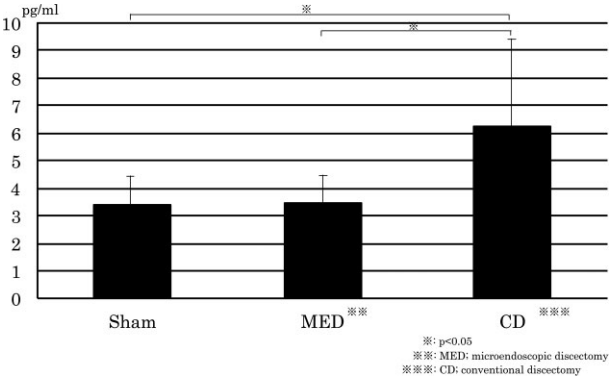

Figure 6 Serum TNF-α levels. Serum TNF-α levels were not significantly different between the sham and MED groups, but the conventional discectomy group was significantly higher than both the sham and or MED groups. CD, conventional disectomy; MED, microendoscopic discectomy; TNF, tumor necrosis factor.

In the MED group, production of IL-6 was confirmed in and around the paraspinal muscles at the site at which the tubular retractor was inserted, as well as in the periosteum and around the part of the vertebral arch that would have been in contact with the tubular retractor (Figure 7d).

In the conventional discectomy group, production of IL-6 was confirmed in wide areas around the bone and in the paraspinal muscles (Figure 8c).

The areas where IL-6-producing cells were confirmed were $4.12–4.93 \times 10^6$ μm^2 (mean, 4.57×10^6 μm^2) in the conventional discectomy group and $2.56–3.55 \times 10^6$ μm^2 (mean, 3.10×10^6 μm^2) in the MED group. The areas were significantly smaller in the MED group (Figure 9). Furthermore, the numbers of IL-6-producing cells per unit area were 35.0–55.0 cells/1.0×10^5 μm^2 (mean, 46.2 cells/1.0×10^5 μm^2) in the conventional discectomy group and 22.0–36.0 cells/1.0×10^5 μm^2 (mean, 29.1 cells/1.0×10^5 μm^2) in the MED group. There were significantly fewer IL-6-producing cells in the MED group (Figure 10).

Discussion

The gold standard surgical approach for lumbar disc herniation is the Love method (conventional discectomy). However, since the first report by Foley and Smith in 1997, MED has become common as a less invasive surgical procedure in Japan (1).

Kawaguchi *et al.* reported that damage to the paraspinal muscles is dependent upon the retraction

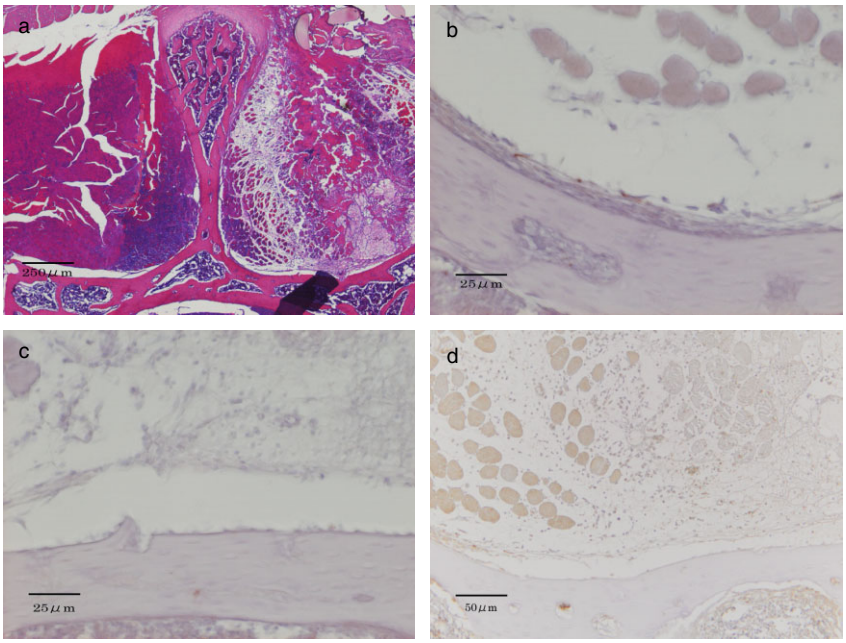

Figure 7 MED group. (a) HE staining (× 40). (b) GAP-43 staining (× 200). The remaining site comprised of periosteum. (c) GAP-43 staining (× 200). The site at which the periosteum disappeared. (d) IL-6 staining (× 400). Degeneration of the paraspinal muscles and inflammatory cellular infiltration were observed along the site of insertion of the tubular retractor. The presence of nerve terminals was confirmed by GAP-43 staining in the same sites at which the remaining periosteum had been confirmed by HE staining. IL-6-producing cells were found in and around the paraspinal muscles where the tubular retractor had been inserted. GAP-43, anti-growth associated protein-43; HE, hematoxylin–eosin; IL-6, interleukin-6; MED, microendoscopic discectomy.

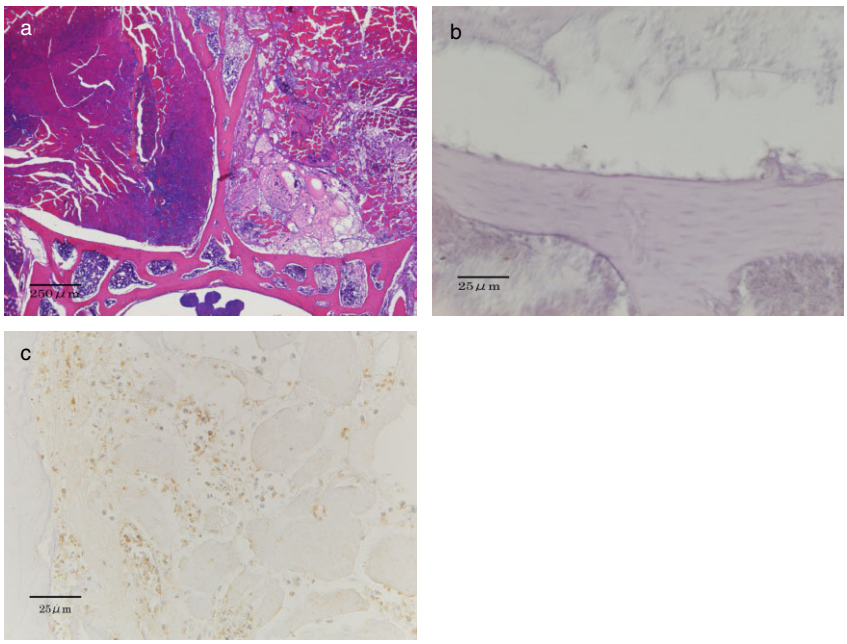

Figure 8 Conventional discectomy group. (a) HE staining (× 40). (b) GAP-43 staining (× 200). (c) IL-6 staining (× 400). A wide range of muscle degeneration was observed, and the periosteum disappeared completely at the sites where the paraspinal muscles were separated from the spinous process to the vertebral arch. In the sites where the periosteum had disappeared, there were no GAP-43-positive cells, and production of IL-6 was confirmed in wide areas around the bone and in the paraspinal muscles. GAP-43, anti-growth associated protein-43; HE, hematoxylin–eosin; IL-6, interleukin-6;

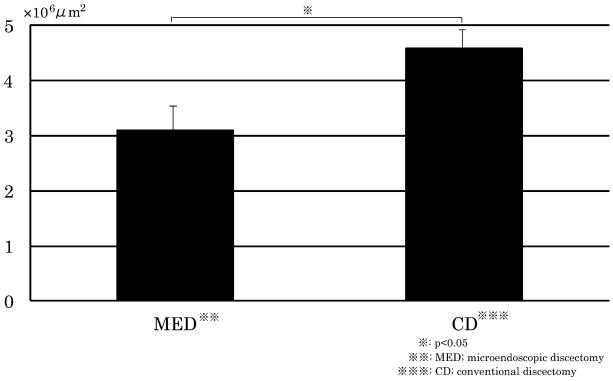

Figure 9 The area of the sites at which IL-6-producing cells were observed in the tissue was significantly smaller in the MED group. CD, conventional disectomy; IL-6, interleukin-6; MED, microendoscopic discectomy.

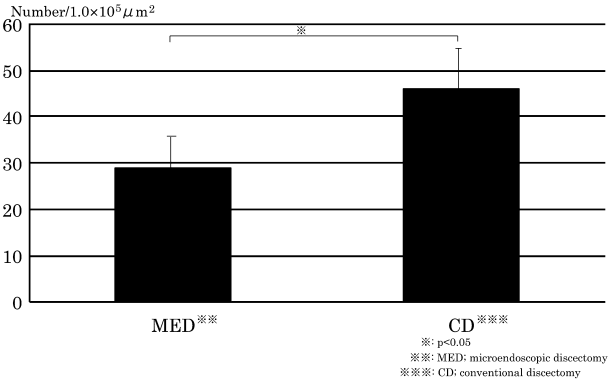

Figure 10 The numbers of IL-6-producing cells per unit area were significantly lower in the MED group. CD, conventional disectomy; IL-6, interleukin-6; MED, microendoscopic discectomy.

duration and pressure (13). MED was developed in order to reduce this muscle damage. In contrast to conventional discectomy, MED does not separate the paraspinal muscles from the vertebral arch during the approach twoard the vertebral arch, and rather than being separated, the paraspinal muscles are split and dilated with a tubular retractor. Thus, MED is expected to reduce surgical stress.

As the popularity of MED has increased, various studies have been conducted to evaluate the invasiveness of this method. Dezawa *et al*. reported that IL-6 in the spinal fluid and blood were useful biomarkers of invasiveness (14). Sato *et al*. also reported that, based on blood natural killer cell activity reflecting the physiological stress due to surgeries, patients undergoing MED recovered from postoperative stress more rapidly (15).

Some researchers consider MED to be less invasive because it does not involve the separation of the paraspinal muscles and periosteum from the spinous process and vertebral arch. However, it is difficult to compare the degree of invasiveness based only on the differing methods of approach between conventional discectomy and MED. Therefore, we examined the invasiveness in rats by changing the methods of approach from the paraspinal muscles to the periosteum alone, while standardizing the other techniques.

The ethological examination in rats was performed using the von Frey test. In the von Frey test, escape

behaviors are generally observed after the footpad has been stimulated (11,12). However, in this study, in order to clearly observe the threshold values of pain, we observed escape behaviors after the fascia was stimulated.

In the blood biochemical examination, CRP and CPK levels were investigated as indices of surgical stress. It is generally believed that when tissues are damaged, intracellular or stromal immune complexes and endotoxins are released into the circulation; for example, IL-6, IL-1, and TNF-α are released from macrophages and monocytes (16). Because IL-6 is not involved in spinal surgeries, it is often used for the evaluation of postoperative pain (17–23). Therefore, we also measured serum IL-6 and TNF-α, which are inflammatory cytokines.

In histological examination, degeneration of the paraspinal muscles due to surgical stress was measured through HE staining, and the production of inflammatory cytokines in paraspinal muscles was evaluated through IL-6 staining.

Damage to the nerve terminals in the periosteum was evaluated using GAP-43 staining (24,25).

Three types of examination were performed: ethological, blood biochemical, and histological. The ethological examination revealed that the threshold pain values in the MED group were similar to those in the sham group, but were higher than those in the conventional discectomy group.

The blood biochemical examination showed elevated serum CPK and TNF-α in the conventional discectomy group relative to the MED group.

The histological examination indicated that there were fewer IL-6-producing cells in the paraspinal muscles of the MED group than of the conventional discectomy group. Based on the results of the GAP-43 staining, it also indicated that there was less invasion to the nerve terminals on the periosteum.

In this study, we compared the invasiveness to the paraspinal muscles and the periosteum via the different methods of approach, but we did not compare conventional discectomy and MED themselves, including actual discectomy operations. However, differences in the methods of approach are considered to be important in reducing postoperative pain. In the future, to advance minimally invasive operations for discectomy, decompression, and spinal fusion, we believe that it will be necessary to develop new methods of approach that can minimize both separation of the periosteum and damage to the paraspinal muscles.

One limitation of this study is that carrageenan was used to induce pain. However, we did not evaluate pure postoperative pain because rats have high pain thresholds and differences in the methods of approach cannot necessarily reveal differences in pain threshold values. However, as both groups were treated with carrageenan, we demonstrated that the method of approach to the vertebral arch with MED induced less postoperative pain than conventional discectomy.

Acknowledgment

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