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Comparison of Three Methods of Drug Delivery in the Rat Lumbar Spinal Subarachnoid Space

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ABSTRACT

This study aimed to compare three most widely used surgeries for experimental lumbar spinal drug delivery in rats. The comparison consisted of assessing the effects of the surgeries and evaluating the deficits produced by these three methods. Sprague Dawley rats underwent acute needle puncture, chronic catheterization via laminectomy or nonlaminectomized catheterization. Body weight changes were measured, animals' general and neurological conditions were observed after surgeries, and motor function was examined by Rota Rod test both prior to and post surgery. Furthermore, nociceptive tests were performed to assess the animals' nociception; hematoxylin, and eosin staining of lumbar spinal cord tissue was performed to evaluate local inflammation caused by surgeries; and both lidocaine paralysis detection; and toluidine blue dye assay were used to confirm the exact location of the catheter. Both needle puncture and catheterization via laminectomy had relatively low success rate of surgery and induced various neurological signs; more severe motor dysfunction, hyperalgesia, allodynia, and local inflammation. Nonlaminectomized catheterization had a higher success rate of surgery, and induced only mild agitation, slight cerebral spinal fluid leakage, mild sensory and motor abnormalities, and minimum pathology in the lumbar spinal cord. The nonlaminectomized catheterization used in this study induces a phenotype of less detectable effects on the animal's behavior and is well-tolerated compared to the acute needle puncture and laminectomized catheterization that are widely used in the literature. Nonlaminectomized catheterization is a safe, accurate and effective way for lumbar drug delivery in rats. Anat Rec, 295:1212–1220, 2012. ©2012 Wiley Periodicals, Inc.

Key words: puncture; laminectomy; catheterization; lumbar subarachnoid space

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Two main techniques of intrathecal drug administration are used in various animal models for pain study, that is, acute needle puncture, and chronic catheter implantation into the subarachnoid space respectively. Needle puncture (Mestre et al., 1994), although extremely valuable, is technically challenging. Catheterization, as first described by Yaksh and Rudy (Yaksh and Rudy, 1976) and later on modified by LoPachin et al. (1981), has become the most extensively used method for rat intrathecal drug delivery (Storkson et al., 1996). Both drug administration methods are associated with individual advantages and disadvantages. The application of needle puncture for example results in the inability to conduct *post hoc* verification of the injection site. In addition the puncture itself can cause stress-induced changes in the Hypothalamic-Pituitary-Adrenal axis (Tsigos and Chrousos, 2002). With regard to catheterization, detachment of the PE tube (Asato et al., 2001), neurological impairment (Gonzalez-Darder et al., 1989; Storkson et al., 1996), and a high occurrence of spinal cord trauma or inflammation (Jones and Tuszynski, 2001), are still difficult to avoid.

Poon et al. (2005) defined successful subarachnoid surgeries by three criteria. First, animals were observed for no motor and sensory abnormalities, no leakage of CSF, no bladder dysfunction or no self-mutilation. Second, rats were assessed for normal body weight gain during the time interval between surgery and experiment. Third, no leakage of the administered solution after intrathecal application were observed.

There's a lack of uniformity in surgeries of acute needle puncture, catheterization via laminectomy, and nonlaminectomized catheterization among different laboratories and researchers, despite the need to keep these variables as constant as possible to accurately compare the various surgeries. As such, a detailed comparison between the different methods is difficult to obtain from the literature, while a head-to-head comparison would be a useful addition. Therefore, this report aims to compare the three previously mentioned surgeries under strict conditions to standardize the surgical and test procedures and validate the optimal choice.

MATERIALS AND METHODS

Materials

Animals and catheter. A total of 85 male SD rats (Tongji Medical College Animal Center, Huazhong University of Science and Technology) weighting 250–300 g were used. The animals were housed at 23°C ± 2°C with *ad libitum* access to food and water, while maintaining a 12 h light/dark cycle. After lumbar surgeries, the rats were housed in individual cages. Our study was carried out according to the Ethical Guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and was approved by the local animal research ethics committee.

The polyethylene 10 (PE-10) catheters (ALZET Osmotic Pumps, Cupertino, CA) was provided by the Department of Anesthesia, Union Hospital, Wuhan, outer diameter 0.5 mm. The catheter was cut to 8.0 cm. A knot was made by silicon glue 2.0 cm from one end. The dead space in the catheter was 8 ± 1 μL. The 1% heparin (10 mg kg^{-1})-saline was injected through the catheter to anticoagulation and check for leakage.

Methods

Three methods of drug delivery in the lumbar subarachnoid space. The rats were anesthetized by 0.8 mL kg^{-1} Hypnorm i.m. (Janssen, High Wycombe, Buckinghamshire, UK) and 0.4 mL kg^{-1} Dormicum s.c. (Roche, Almere, The Netherlands). The specific procedures to achieve the following: Step 1, retrieve the animal from its cage, place it in a tared container, and determine its weight, return the animal to its cage; Step 2, calculate the appropriate dosage of the chosen anesthetic (0.8 mL kg^{-1} Hypnorm i.m. and 0.4 mL kg^{-1} Dormicum s.c.), fill 1-mL syringes with selected anesthetic, respectively; Step 3, manually restrain the animal, performing Hypnorm injection in the caudal thigh muscles first, after the animal sleep, injecting Dormicum subcutaneously in the area of the dorsal skin using a 20-G needle. All instruments and implantable parts were sterilized, and the operated region was clipped and sterilized with iodine tincture and 75% alcohol. A sterilized soft cloth is placed over the head and upper body of each rat.

For acute needle puncture, sterile drapes were placed over the upper body of rats, and then grip the rat firmly by the hip bones with one hand. With the other hand, opened up the intervertebral space by pushing the spinal column of the rat to create a slight curve. Animals were briefly injected at the L4-5 interspace using a 1.5-in. 27-gauge needle connected to a 50 μL Hamilton syringe. Correct dura puncture and position of the tip of the needle was verified by a reflexive flick of the tail or formation of an "S" by the tail. Once the reflexive tail flick or "S" shape has been observed (Mestre et al., 1994).

For catheterization via laminectomy, first, the back is approached through a 2-cm long incision in the midline of the back, and the paraspinal muscles were retracted subperiostically. After the spine is approached, L4-L5 vertebral laminae were exposed. Furthermore, a total L4 laminectomy was performed and the ligamentum flavum and peridural fat tissue were cleared away from the surgical site, allowing visualization of arachnoid and spinal cord tissue. Finally the catheter was flushed with sterile saline and inserted 2 cm rostrally. Correct subarachnoid placement of the catheter was verified by the identical tail or paw flick with need puncture. The catheter is then secured to the cervico-auricular muscle with suture, locked with artificial CSF, and sealed with heat. The remaining catheter is tunneled subcutaneously to a neck incision and secured in place with a wound clip. The lumbar midline skin incision is then closed with wound clips (Hinton et al., 1995).

As to nonlaminectomized catheterization, a dorsal midline incision is made in the skin at the level of L3-L6 vertebrae, the L4-L5 interspinous ligament was carefully cut to expose the space between L4 and L5 vertebrae. A small puncture is made through the membrane with a needle and the catheter was inserted through the puncture hole and pushed cautiously under the surgical microscope through the intervertebral space dura. When the sign of dura penetration (sudden movement of tail or the hindlimb) was observed, the catheter was inserted cephalad into the subarachnoid space 2.0 cm upward. The catheter is then secured to the cervico-auricular muscle with suture, locked with artificial CSF and sealed with heat. The remaining catheter is tunneled subcutaneously to a neck incision and secured in place

with a wound clip. The lumbar midline skin incision is then closed with wound clips (Gonzalez-Darder et al., 1989; Sakura et al., 1996a).

Body Weight Measurement

The rat's body weight (BW) was measured by net weigher scale before, and at 1, 2, 3, 5, 7, 10, and 14 days after the surgical manipulations. A total of 24 rats was randomly assigned to four groups (normal control, acute needle puncture, catheterize via laminectomy, and non-laminectomized catheterization), thus resulting in six rats per group. Each animal was weighed three times.

General Behaviors and Neurological Assessments

To compare the influence of the surgeries on animals (15 rats per surgery group, 45 of the total rats were used), general behavior of the animal and neurological deficits were assessed 2 days after surgery. General behaviors included agitation, allodynia, and catalepsy. Agitation was judged by spontaneous vocalization or restlessness. Allodynia was examined by escape behavior or a vocalization response evoked by lightly stroking the flank of the rat with a plastic stick. Catalepsy was tested by placing the fore-paw slightly higher than the hind-paw. The intensity of catalepsy was usually assessed by observing the duration of the longest immobile episode within a 2-min period. If catalepsy was pronounced, observation was continued till the first movement occurred. The longest period of complete immobility encountered was 10 min. Finally, the animals were inspected with respect to some typical neurological signs including postoperative morbidity, leakage of CSF, paraplegia, bladder dysfunction, and self-mutilation. Postoperative morbidity was defined by decrease of food and water intake, messy hair and less movement. Leakage of CSF was confirmed by direct observing of the clear liquid exudation from the wound or the outer part of the implanted catheter (Ozisik et al., 2006). Paraplegia was identified by the sensation and movement disappearance of the hindlimb and tail, bladder dysfunction was determined by frequency of urination, incontinence and/or anuria (Gevaert et al., 2006). Self-mutilation was evaluated at the daily basis. Three-grade scale (nonintact; mild-intermittent mutilation (permanent licking, skin depilation and denuding, scratching with visible bleeding); severe-removal of the toe parts up to full forelimb autotomy) based on qualitative pattern was used for the evaluation of self-mutilation (Kriz et al., 2006). The prevalence of general behaviors was expressed by degrees of response, while neurological deficits were represented by frequency of occurrence.

Rota-Rod Test

At 0 (before surgery), 1, 3, 7, and 14 days after surgery, motor function of the animals (24 rats in total and each group has 6 rats) for bodyweight measurements was investigated using a rota-rod treadmill for rats (Ugo Basile, Italy), under the accelerating rotor mode (10 speeds from 4 to 40 rpm for 5min). The interval from when the animal mounted the rod to when it fell off was recorded as the retention time (RT), and the population of operated rats that walked for 300 sec on the accelerating rotating rod was recorded as the walking survivor (WS) (Kitamura et al., 2005; Yanagisawa et al., 2008). The animals were trained for 2 days, three trials per day, before surgery and the mean duration on the rod was recorded to obtain stable baseline values. Performance on the rota-rod test was measured three times a day in the following 2 weeks after lumbar surgeries. The RT was presented as mean ± SEM, the WS was presented as absolute percentage of the total walking animals.

Nociceptive Behavioral Tests

After the rota rod experiments, the animals' (24 rats in total and each group has 6 rats) nociception were examined. Thermal hyperalgesia was assessed by exposing the mid-plantar surface of the hindpaw to a beam of radiant heat through a transparent glass surface using a plantar analgesia meter for paw stimulation (Ugo Basile, Italy), as described previously (Hargreaves et al., 1988). Mechanical allodynia was inspected by placing an animal on an elevated mesh floor. Subsequently, the tactile threshold was measured by using an electronic von Frey anesthesiometer (Ugo Basile, Italy) applied to the plantar surface of the hindpaw (Pitcher et al., 1999). In addition the force (g) needed to produce a paw withdrawal response was tested. The hindpaw withdrawal thermal latency (HWTL) and hindpaw withdrawal mechanical latency (HWML) were recorded three times with a 5-min interval, for both left and right hindpaws as the time taken from the onset of radiant heat and hand-held force transducer stimulation to withdrawal of the hindpaw. Both the thermal and mechanical pain measurements were performed on Day 0 (before surgery), 1, 3, 7, and 14 after surgery. A mean value of these three consecutive measurements was taken for each paw.

Tail Flick Test

The antinociception of the punctured or catheterized rats was assessed in the tail flick test on Day 0 (before surgery), 1, 3, 7, and 14 after surgery (Cecchi et al., 2008). After nociceptive behavioral tests, rats (24 rats in total and each group has 6 rats) were placed on the tail-flick apparatus to habituate them to the procedure. A radiant heat source (75-w bulb) was focused on 5 cm from the tip of the tail, an abrupt flick of the tail secondary to the thermal stimulus was sensed and the time from the onset of the heat stimulus to withdrawal of the tail from the heat source recorded. The latency to tail flick (TFL) was recorded automatically (Ugo Basile, Italy). The cut-off value was set at 20 s.

Hematoxylin and Eosin Staining of the Lumbar Spinal Cord

To determine whether acute needle puncture or chronic catheterization induced a significant local inflammatory response, lumbar spinal cord hematoxylin and eosin H&E staining was performed prior (0 day) and postsurgery at indicated time points (1, 3, and 7 days). Rats were anesthetized with pentobarbital (35 mg kg^{-1}, i.p.) after which the spine was cut *en bloc*, postfixed with 10% formalin,

decalcified, and paraffin embedded. The tissue was sliced at 4 μm thickness and then stained with H&E. H&E stained sections were selected from the L4/5 spinal cord of two animals at each time point per group (8 rats for each time point and 32 rats in total were used) to analyze the inflammation in the spinal dorsal horn. Signals were analyzed under 10× microscopic visual fields (Olympus BH-2). Photographs were taken with a digital camera (Panasonic, DMC-F1, Japan), digitally processed and printed. Images were obtained by a microscopy-digital camera system (TK-C1381EG; Victor Company of Japan Limited, Japan). The number of the nuclei in the spinal dorsal horn were calculated and recorded by using the High Vivid Color Pathological Photo Analyze System (HPIAS-1000; Huazhong University of Science and Technology).

Lidocaine Paralysis Test

After finishing all the behavioral experiments (on Day 14 after surgeries), the free end of the catheter was cut to allow a single injection of 10 μl 2% lidocaine, followed by 10 μL saline. For acute needle punctured rats, a single injection of 10 μl 2% lidocaine was injected directly into the subarachnoid space. Saline was administered as control. Needle puncture and catheter location were confirmed by observing the paralysis of the hindlimb and tail within a few seconds, lasting for at least 10 min.

Toluidine Blue Dye Experiment

Finally, rats were injected intrathecally with 10 μL of 1% toluidine blue (TB) via a syringe connected with the punctured needle or outer part of the PE-10 tube. The rats were anesthetized with pentobarbital (35 mg kg^{-1}, i.p.) and transvascularly perfused with 300 mL of 37°C saline. Subsequently, the spine was removed *en bloc*, the spinal cords were completely dissected and the dye distribution was checked and compared. Ten micro liter of normal saline was injected for the control condition.

Data Analysis and Statistics

Data are reported as means (SD). Changes in RT, BW, HWTL, HWML, and TFL of surgery and control groups were analyzed with repeated measures ANOVA, followed by Dunnett's *post hoc* test. Differences in WS were analyzed with a log-rank test. Differences of inflammation in the spinal dorsal horn among different groups were compared by one-way ANOVA, followed by the Student-Newman–Keuls multiple-range test for *post hoc* assessment. $P < 0.05$ was considered to be statistically significant. The observer was blind to experimental the interventions.

RESULTS

Body Weight Changes After Lumbar Surgeries

After surgery, rats with a lumbar laminectomized catheterization lost BW from Days 1 to 7, with the minimum on day 3 as compared to the normal group at corresponding days (*$P = 0.02$, two way ANOVA). Rats with needle puncture and nonlaminectomized catheterization seemed to maintain normal food and water intake, though a slight but non-significant decrease in BW occurred within the first week compared to the normal group (Fig. 1).

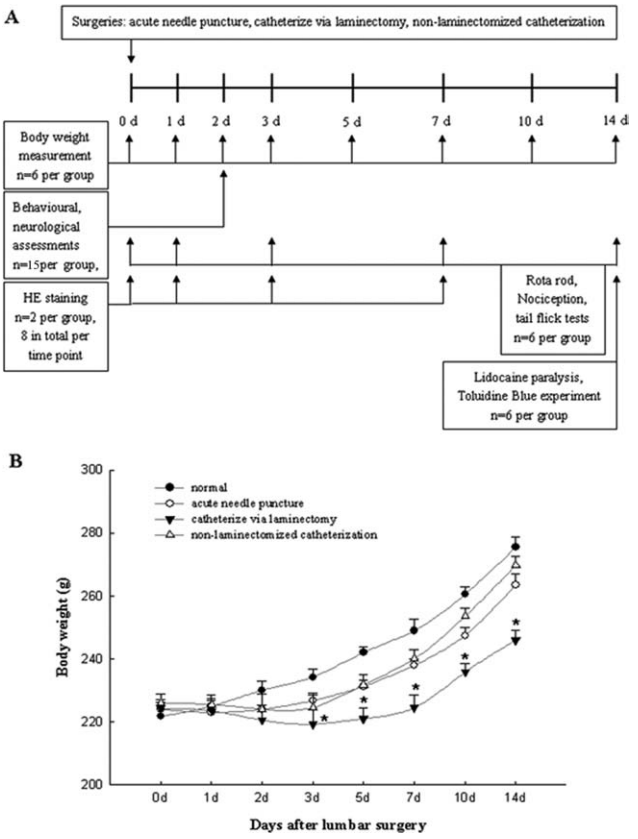

Fig. 1. (**A**) Flow diagram outlining specifically the experimental protocol. (**B**) Body weight development of normal, acute needle puncture, catheterize via laminectomy and nonlaminectomized catheterization groups pre and postlumbar surgeries within 2 weeks.

TABLE 1. Comparisons of postoperative general behaviors among three surgeries

General behaviors	Acute needle puncture	Catheterize via laminectomy	Nonlaminectomized catheterization
Agitation	++	+	±
Allodynia	+++	++	−
Catalepsy	+	−	−

Scores: not response (−), dubious response (±), mild response (+), moderate response (++), and intense response (+++).

Comparisons of General Behaviors and Neurological Signs After Lumbar Surgeries

As displayed in Table 1, acute puncture as well as laminectomy induced obvious agitation, allodynia, and catalepsy. However, nonlaminectomized catheterization did not produce any abnormal behaviors except casual agitation. As summarized in Table 2, in the

TABLE 2. Comparisons of postoperative neurological signs among three surgeries

Surgeries (animal numbers)	Acute needle puncture (15)	Catheterize via laminectomy (15)	Nonlaminectomized catheterization (15)
Death	3	2	1
Leakage of CSF	2	3	1
Paraplegia	4	2	2
Bladder dysfunction	2	0	0
Self-mutilation	0	1	0
Success rate	26.7%	46.7%	73.3%

Values in parentheses represent the total numbers of rats. CSF, cerebrospinal fluid.

nonlaminectomized group, an absolutely low mortality rate of 6.7% (1/15) was observed, and only 13.3% (2/15) of the rats exhibiting flaccid paresis after surgery. Leakage of CSF, either from the wound after surgery or subsequent to intrathecal application of solution during the experiment, was observed in 6.7% (1/15) of the rats. None of them manifested bladder dysfunction or self-mutilation. In total, we achieved a 73.3% (11/15) success rate with the method of nonlaminectomized catheterization, while 26.7 and 46.7% for applying acute needle puncture and laminectomized catheterization respectively.

Effects of Lumbar Surgeries on Motor Function in the Rota-Rod Test

The results showed no difference in the RT (Fig. 2A) among the three groups pre-treatment. In the 7 days following acute needle puncture or catheterization via laminectomy, the RT on the rod markedly shortened compared with the nonlaminectomized catheterization on Days 1, 3, and 7 (#$P = 0.003$, *$P = 0.008$, two way ANOVA). Although the rats receiving a nonlaminectomized catheterization showed a slight reduction in RT on the rod at 7 days postsurgery compared with normal rats, the difference was not significant.

The population of operated rats that walked for 300 sec on an accelerating rotating rod (Fig. 2B) was significantly decreased by needle puncture (open circle, $P = 0.03$) or catheterization via laminectomy (closed triangle, $P = 0.01$), in comparison to the normal rats (closed circle,). However, there is a slight decrease of the WS in the nonlaminactomized group (open triange), but it's not significant as compared to the control group.

Pain Measurements After Lumbar Surgeries

In the thermal pain measurement (Fig. 3A), the HWTL for the normal and nonlaminectomized catheter rats before and after lumbar surgery were very similar, ranging from 8.3 ± 0.9 sec to 9.0 ± 0.6 sec and from 8.4 ± 0.9 sec to 9.1 ± 1.4 sec. No significant difference was seen between the two groups. However, the HWTL was decreased from 8.5 ± 1.3 sec and 8.9 ± 1.2 sec to 4.7 ± 0.4 sec and 3.7 ± 0.5 sec for the rats' hindpaws of acute needle puncture and catheterize via laminectomy respectively. The differences were significant at 1, 3, 7, and 14

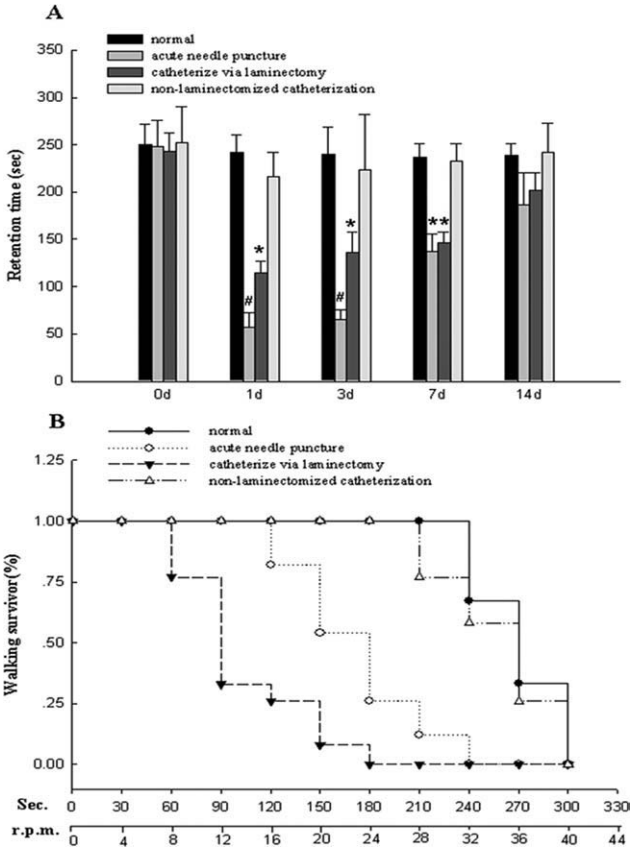

Fig. 2. Effects on motor function in Rota-Rod test in lumbar operated rats by acute needle puncture, catheterization via laminectomy and nonlaminectomized catheterization (**A**) Comparison of endurance on the rotating rod among four groups pre and 2 weeks postsurgery. (**B**) Time-dependent changes in the percentage (%) of walking rats on the rotating rod. Sec., second; r.p.m., revolutions per minute.

days after surgeries among acute needle puncture, catheterize via laminectomy and normal groups (#$P = 0.004$, *$P = 0.03$, two way ANOVA).

Consistent results were also found in the mechanical pain test (Fig. 3B). Although a slight decrease can be seen in the nonlaminectomized group, the difference was not significant as compared with the normal group. The HWML ranged from 26.2 ± 1.4 g to 28.3 ± 2.1 g and 28.2 ± 2.5 g to 23.2 ± 1.9 g for normal rats and catheterized rats, respectively. Whereas the HWML was significantly reduced from 29.5 ± 4.2 g and 27.6 ± 4.6 g to 17.0 ± 0.8 g and 14.2 ± 0.7 g at 1, 3, and 7 days after acute needle puncture and catheterization via laminectomy (#$P = 0.002$, *$P = 0.02$, two way ANOVA).

Tail Flick Test After Lumbar Surgeries

The TFL in control rats was stable, ranging from 10.8 ± 0.7 sec to 11.9 ± 0.3 sec; whereas nonlaminectomized catheterization induced a slight decrease of TFL in the 2 weeks following lumbar surgeries, ranging from 10.6 ± 1.7 sec to 9.0 ± 0.4 sec. The difference was not significant except on Day 1 after surgery. However, in the

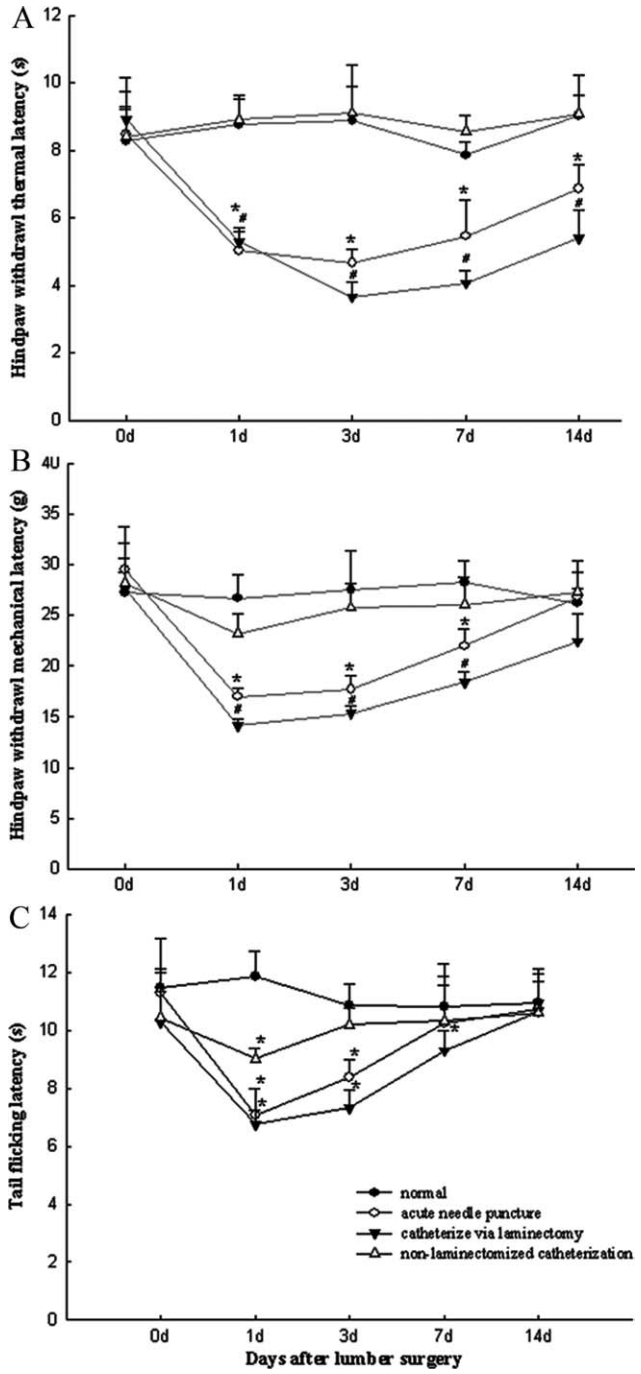

Fig. 3. The alterations of rat hindpaw sensation pre (0 day) and postlumbar surgeries at 1, 3, 7, and 14 days. (**A**) shows the thermal pain measurement, (**B**) represents the mechanical pain assay, and (**C**) displays that the Tail Flicking Latency (TFL) among the normal, acute needle puncture, catheter via laminectomy and nonlaminectomized groups.

needle puncture and catheterize via laminectomy groups, the TFL decreased significantly from 11.3 ± 0.8 sec and 10.3 ± 0.4 sec to 5.1 ± 0.9 sec and 6.7 ± 1.0 sec within 7 days (*$P = 0.03$, two way ANOVA), and was minimal 1 day after surgeries (Fig. 3C).

H&E Staining of L4/5 Spinal Cord After Surgeries

As shown in Fig. 4A, acute needle puncture and catheter via laminectomy caused a clear inflammatory response that was tightly localized to the spinal dorsal horn. Some polymorphonuclear leukocytes showed a marked increase within 7 days after surgeries, while the inflammation did not seem to vary in intensity at the different time points after nonlaminectomized catheterization. In the control group, in contrast to the surgery groups, no increase of leukocytes was observed on the indicated days. Figure 4B illustrates that the number of hematoxylin stained nuclei in the L4/5 spinal dorsal horn significantly increased on 1, 3, and 7 days after surgery both in the needle puncture and laminectomized catheterization groups (#$P = 0.004$, *$P = 0.03$, one way ANOVA). For the nonlaminectomized catheterization group, the inflammation was non-significant except at Day 1 after surgery.

Lidocaine Effects

The positions of needle and catheter were confirmed by injection 10 μL of 2% lidocaine (Anesthesia Department of Tongji Hospital) flushed with 10 μL of saline 2 days after lumbar surgeries. Lidocaine caused 83.3% (5/6) paralysis of the hindlimbs and tails within 15 sec and lasting 10–20 min in both the laminectomized and nonlaminectomized catheterization groups. However, the rate of lidocaine effects in the needle punctured group was 50.0% (3/6). Normal saline was injected as a control and no deficits occurred (Fig. 5).

Spinal Distribution of Toluidine Blue

We compared the spinal distribution of dye after injection of 20 μL 1% TB through a punctured needle, and laminectomized and nonlaminectomized catheters. The twenty microliter injections showed circumferential of the dye spread to the T10 level rostrally and the sacral region of the spinal cord caudally. The rostral and caudal extents of dye spread are shown in Fig. 6. In addition, in the needle puncture group, the tissues' integrity of the lumbar spinal cord was impaired more severely than in the catheter groups.

DISCUSSION

In this study we compared the three scheduled lumbar surgeries of intrathecal drug delivery in SD rats, based on the previously reported criteria (Poon et al., 2005), using a standardized range of tests to allow for a more complete comparison. The tests consisted of the examination of body weight, general behaviors, neurological deficits, motor function, irradiant heat and von Frey assays, tail flick test, HE staining, lidocaine, and dye injection experiments.

Our results indicate a fairly high success rate and avoidance of some concomitant symptoms in the group of nonlaminectomized catheterization as compared to needle puncture and laminectomized catheterization. The most consistent observation was a marked decrease in the morbidity, mortality and neurological deficits associated with catheter placement in the surgery of nonlaminectomized catheterization. This decrease in the

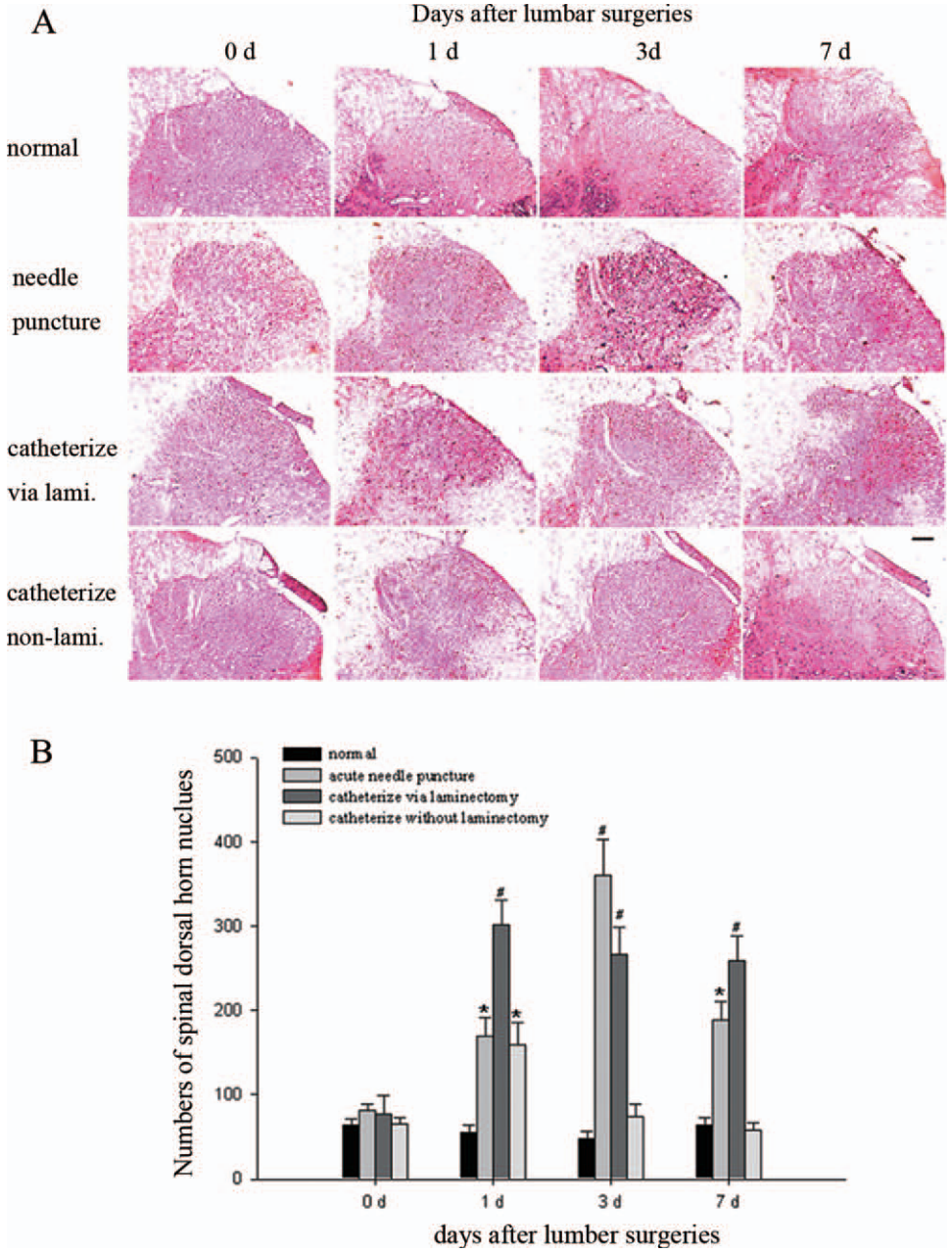

Fig. 4. (**A**) present the H&E staining of L4/5 spinal cord sections (4 μm) at 0, 1, 3, and 7 days after lumber surgery, scale bar = 100 μm. The numbers of nuclei (dark blue particles in the picture) on spinal dorsal horn are calculated and analyzed, the histogram was displayed in (**B**).

degree of tolerance to the surgeries and the increase in the surgery-causing symptoms were found in the punctured and laminectomized groups. The differences among the three surgeries most probably arises from the following reasons. Firstly, lumbar needle puncture is technically difficult. The angle and depth of the needle's insertion is variable because of the individual difference among the animals, and the tract of the needle is flexible and the needle is prone to bending, making control of the insertion more difficult. Secondly, laminectomy involves removal of not only a portion of the vertebral column itself, but also the muscles attached to (or crossing) the resected bone and ligaments. Exposure of the spinal cord can be accompanied by serious complications such as spinal dislocation. Furthermore, local ablation of the vertebra frequently results in some hemorrhage and inflammation which causes formation of scar tissue around the tip of the catheter (Yaksh et al., 1999). Finally, lumbar insertion through the intervertebral space using a relatively safer trick not by directly rough puncture and traumatic laminectomy, reduced neurological and general complications. This was also consistent

with the report that the lumbar indwelling catheter method produced reduction in neurological deficits, normal weight gain, and reduced recovery time before experimentation (Storkson et al., 1996).

The thermal and mechanical hypersensitivity within 2 weeks displayed in pain behavior were observed in punctured and laminectomized groups when compared with the nonlaminectomized and normal groups. Apart from a light hyperalgesia on postoperated Day 1 with the nonlaminectomized catheterization, the response to the irradiant heat and von Frey stimulation remained relatively stable. These results may be explained by the report that repeated puncture can result in stress-induced hormone changes, and may indirectly influence the nociceptive sensitivity of the experimental subjects. In addition (Tsigos and Chrousos, 2002), the restraint during the puncture manipulation, that is simply put in a restraint, while the needle was placed in the subarachnoid space, appears to be stressful to the rat. Moreover, there is evidence that indicates that stress and inflammation causes generalized hyperalgesia by enhancing pronociceptive effects of immune mediators (Khasar et al., 2008).

We found that a marked inflammation in the L3/4 dorsal spinal cord occurred within 7 days in the groups of puncture and laminectomized catheterization, while the nonlaminectomized catheterization did not cause significant inflammation in the lumbar enlargement portion except on Day 1 after surgery. A previous study demonstrated that syringe needles and catheters most often used for intrathecal administration can elicit a substantial inflammatory response (Yaksh et al., 1999; Xu et al., 2006). Our results are not completely consistent with this report. This difference might be a result of the different phase of inflammation chosen for observation. However, there is still evidence that less histologic abnormalities and less inflammation of the spinal cord could be obtained using catheterization (Sakura et al., 1996b).

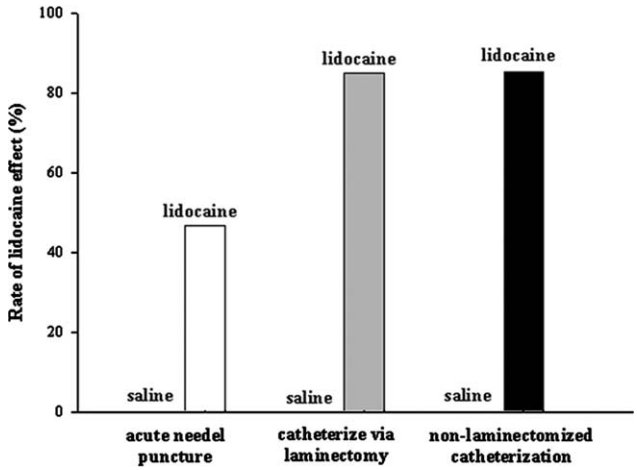

Fig. 5. The histogram shows the rate of lidocaine effects among three surgeries. Catheterization without laminectomy group is markedly higher than the punctured and laminectomized group. The rate was presented as absolute percentage.

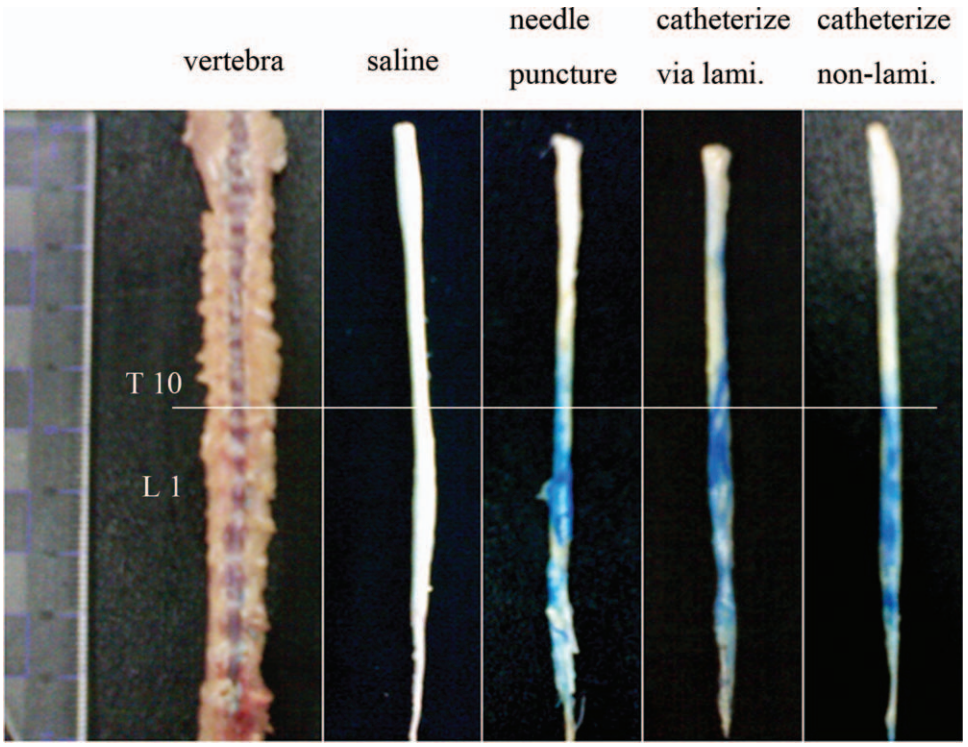

Fig. 6. The photo shows the distribution of dye after intrathecal TB injection through the ways of needle puncture, laminectomized catheter and nonlaminectomized catheter.

One useful method to confirm needle puncture or catheter placement is to inject lidocaine. Motor paralysis should ensue within 15 sec and resolve within 20–30 min (Hargreaves et al., 1988). Another more accepted measure is to inject dye, sacrifice the subject, and examine the spinal cord for location of injected dye as well as catheter tip placement. With our observation, both experiments produced consistent results among the three methods. Repeated daily injection of 2% lidocaine caused various degrees of paralysis in rat's hindlimb and tail. In terms of anesthetic rate, both administration method using an implanted catheter, except injection through puncture, resulted in a preferable paralysis effect. The lower number of paralyses in the punctured group compared to other groups may result from an incomplete needle insertion or drug injection into the paraventebral muscles or outside the spinal cord. In addition, the rats injected via nonlaminectomized catheterization recovered faster than laminectomized animals from the drug and behaved normally. With regard to the dye injection, only the successful surgeries obtained relatively precise staining area, and all the injection dye spread to the sacral regions. This result is consistent with the previous study that a 20 μL injection spreads to T13-L1 and completely to the sacral and cauda equine regions (Xu et al., 2006). This indicates the precise needle or catheter position, and also can be injected in exactly the same location as the lidocaine. However, the impaired tissue integrity occurred more frequently in the needle punctured group than in the catheterization group, which may explain the former motor and sensory experimental results.

In conclusion, without notable exceptions, it appears that all three methods of lumbar spinal intrathecal administration produce different postoperative side effects. In case of acute needle puncture and laminectomized catheterization, controlling for the insertion and operation is somewhat more challenging. The method of nonlaminectomized catheterization on the other hand, avoids the high-precise needle puncture skills and uneasily controlled operations. Nonlaminectomized catheterizaiton also results in minimal morbidity and mortality, less motor dysfunction and hypersensitivity, and less local inflammation. Therefore with some practice, the facilitated method of chronically implanting a catheter into rats' lumbar subarachnoid space without laminectomy represents a highly replicable, site-specific and reliable method for accessing lumbar spinal cord functioning. Comparison among the three surgeries should provide a useful assessment to currently available techniques for the spinal drug delivery.

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