Laboratory Report

A rat model of chronic lumbar epidural catheterisation

Tomoki Nishiyama MD PhD

Purpose: There are no rodent models of chronic epidural catheterisation which can induce clinically relevant analgesic effects of morphine (potency ratio of intrathecal:epidural = 10 to 30:1). The purpose of this study was to investigate a new rat model of chronic epidural catheterisation by comparing the analgesic effect of epidural and intrathecal morphine.

Methods: In Sprague-Dawley rats, for epidural catheterisation, a skin incision and muscle dissection were made at T₁₃ level in the midline. The intervertebral ligament was cut to insert an epidural catheter (polyethylene tube; outer diameter 0.14 mm) 2 cm caudally. The distal end of the catheter (0.61 mm) was passed sc to exit in the dorsal neck area. For intrathecal catheterisation, the atlanto-occipital membrane was incised to insert a catheter (0.61 mm) 8.5 cm caudally. The effects of morphine (1, 3, 10, 30, or 100 µg·10 µl⁻¹ intrathecally, and 30, 100, or 300 µg·5, 10, or 20 µl⁻¹ epidurally) on thermal escape latency were investigated by a hot box test. Behaviour and motor function were also tested.

Results: A volume of 20 µl induced greater analgesic effect than 5 and 10 µl epidural administration. The ED₅₀ of epidural morphine (94.1 µg) was 30 times more than that of intrathecal morphine (3.1 µg).

Conclusion: A new rat model of chronic epidural catheterisation is described, which provides a similar analgesic epidural:intrathecal potency ratio for morphine to that in humans when morphine is administered in a volume of 20 µl.

Objectif : Il n'y a pas de modèle animal, chez les rongeurs, pour la cathétérisation péridurale chronique qui puisse induire les effets analgésiques pertinents de la morphine (coefficient de puissance dose intrathécale:péridurale = 10 à 30:1). L'objectif de cette étude était de rechercher un nouveau modèle animal (utilisant des rats) de cathétérisation péridurale chronique en comparant l'effet analgésique de la morphine péridurale et de la morphine intrathécale.

Méthode : Chez des rats Sprague-Dawley, on a procédé à une cathétérisation péridurale en faisant une incision de la peau et une dissection musculaire au niveau de T₁₃ sur le plan médian. Le ligament intervertébral a été coupé pour insérer le cathéter péridural (tube de polyéthylène ; diamètre extérieur de 0,14 mm) 2 cm en direction caudale. L'extrémité distale du cathéter (0,61 mm) a été insérée sc pour ressortir dans la nuque. Dans le cas de la cathétérisation intrathécale, la membrane atlanto-occipitale a été incisée et on a inséré un cathéter (0,61 mm). Les effets de la morphine (1, 3, 10, 30 ou 100 µg·10 µl⁻¹ intrathécale, et 30, 100 ou 300 µg·5, 10 ou 20 µl⁻¹ péridurale) sur la latence d'échappement thermique ont été étudiés selon la technique de la boîte chauffante. Le comportement et la fonction motrice ont aussi été étudiés.

Résultats : Un volume de 20 µl a provoqué une analgésie plus importante que 5 et 10 µl par administration péridurale. La ED₅₀ de la morphine péridurale (94,1 µg) a été 30 fois plus élevée que celle de la morphine intrathécale (3,1 µg).

Conclusion : On a décrit un nouveau modèle animal (rat) qui fournit un coefficient de puissance analgésique péridural:intrathécal similaire à celui qu'on retrouve chez les humains quand la morphine est administrée en un volume de 20 µl.

From the Department of Anesthesiology, The University of Tokyo, Faculty of Medicine, Tokyo, Japan. Present affiliation: Harbor/University of California, Los Angeles Medical Center Torrance, CA, USA.

Address correspondence to: Tomoki Nishiyama MD PhD, 1436 Brett Place #24, San Pedro CA 90732 USA. Phone/Fax: 310-519-8298 (Home); Phone: 310-222-3861; Fax: 310-782-1467 (Work).

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EPIDURAL drug administration is widely used in pain management. A reliable experimental animal model of epidural drug administration is of increasing importance. However, there are no standard rodent models of chronic epidural catheterisation. Previous models required four to seven days for recovery from surgery.¹⁻³ However, fibrosis develops at the extremity of the catheter so rapidly, that it can obstruct it completely only 10 days after catheterisation.⁴ This occlusion might render access of morphine into the epidural space more difficult and, thus, the potency ratio of intrathecal: epidural morphine would be similar.^{4,5} However, in humans, the potency ratio of intrathecal:epidural morphine is about 10 to 30:1.⁶⁻¹⁰ I developed a new rat model of chronic epidural catheterisation with a less invasive technique than that used in previous models. I investigated the analgesic effects of epidurally administered morphine using this model and compared the effects with those obtained using well established model of intrathecal catheterisation.

Methods

Experiments were carried out according to a protocol approved by the Institutional Research Committee.

Catheter preparation

The epidural catheter was made of a 2 cm small polyethylene tube (PE-5; Clay Adams, Parsippany, NJ, outer diameter: 0.14 mm) heat-melted to a 12 cm polyethylene tube (PE-10; Clay Adams, Parsippany, NJ, outer diameter: 0.61 mm). The first knot was made at the connection between the PE-5 and PE-10 tubes by heat-melting the two tubes. The second knot was made on PE-10 at 5 mm distal to the first knot by tying the tube without occlusion. The knot was covered with acrylic dental cement. The dead space in the catheter was $8 \pm 1 \mu\text{l}$ (mean $\pm$ SD, $n = 8$).

The intrathecal catheter was made of a 12 cm polyethylene tube (PE-10). A knot was made by tying the tube with acrylic dental cement 8.5 cm from one end.¹¹ The dead space in the catheter was $7 \pm 1 \mu\text{l}$ ($n = 8$).

Animal preparation

Sprague-Dawley rats (300-350 g; Harlan Industries, Indianapolis, IN) were anaesthetized with halothane 4% in an anaesthesia induction box. Upon loss of responsiveness and spontaneous movement, the rat was removed from the induction box and continuously anaesthetized with halothane 2% in air-oxygen mixture with spontaneous respiration.

For epidural catheterisation, the dorsal thoracolumbar spinal region was clipped and sterilized with alcohol and betadine. A 1 - 2 cm midline skin incision was made at the most prominent thoracic spinous

process, T₁₃. Muscles were bluntly dissected from the vertebrae and retracted to expose the intervertebral space. The intervertebral ligament was carefully cut and an epidural catheter was inserted into the epidural space 2 cm caudally. No hole or groove in the bone was made, no bone was dissected, and no glue was used. The catheter tip was located at the L₃₋₄ level. The first knot was placed in the space between the two vertebrae, the second knot was covered by muscle, and the longer portion was passed *sc* to exit the dorsal neck area. No suture or glue was used to fix the catheter. Fluid-free aspiration ensured that the catheter was not placed intrathecally. The catheter was flushed with sterile saline, and no leaks were observed at volumes up to 50 μl . The catheter tip was plugged with a 28G steel wire. The muscles and fascia were sutured with 3-0 Vicryl™ then the skin was closed using 3-0 silk. Inhalation of halothane was stopped and the animals were observed during recovery in a warm box. Only the rats with no motor disturbance on the second post-operative day (98% in total catheter implanted rats) were used for the study. All animals were sacrificed with an over-dose of pentobarbitone (100 mg·kg⁻¹) after the study and the spinal column was removed with the catheter to verify the position of the catheter tip. Data from the rats with the catheter not in the epidural space (5%) were excluded from this study.

Intrathecal catheters were implanted according to the method described by Yaksh and Rudy.¹¹ Briefly, under halothane anaesthesia, the occipital area was clipped and sterilized with alcohol and betadine, a 1 cm midline skin incision was made. An 8.5 cm polyethylene catheter was advanced caudally through an incision in the atlanto-occipital membrane, to the thoracolumbar level of the spinal cord. The external part of the catheter was tunnelled *sc* to exit on top of the skull and plugged with 28G steel wire. Rats with normal motor function and behaviour were used five to seven days after surgery.

Body weight measurement

Body weight was measured before, and at 1, 2, and 6 days after catheter implantation ($n = 20$ in both epidural and intrathecal catheterisation).

Drugs and injection

To assess the epidural volume producing hind-paw analgesia, 5, 10, or 20 μl normal saline, or morphine (morphine sulphate, opioid agonist; Merck, Sharpe and Dohme, West Point, PA) 30 μg , 100 μg , or 300 μg were administered epidurally two days after catheter implantation. Each group included seven rats. Then, according to the data, 20 μl were used for the other studies of epidural administration.

For intrathecal injection, normal saline, morphine 1, 3, 10, 30, or 100 µg were administered in 10 µl five to seven days after catheter implantation. Each group included 10 rats.

Morphine was dissolved in normal saline so that each volume contained the desired quantity of the agent. After epidural or intrathecal drug injection, the catheter was flushed by injection of 10 µl normal saline. A micro injector syringe was used for all injections.

Nociceptive test

All animals were tested for acute thermal nociceptive response using a hot-box test.¹² The rats were placed in a clear plastic cage on an elevated floor of clear glass. A radiant heat source (halogen projector lamp CXL/CXP 50 W 8 V, Ushio, Tokyo, Japan) was contained in a movable holder placed beneath the glass floor. The aperture of the radiant heat source was 4 mm in diameter, and the bulb intensity was controlled by a constant voltage source. The interior of the box under the animal was prepared with a heat source such that the under plate temperature was maintained at 30°C. The under floor heat source was then positioned such that it focussed at the plantar surface of one hind paw where it was in contact with the glass. The calibration of the thermal test system was such that the average response latency in normal untreated rats measured before the initiation of an experiment, was approximately six seconds. At the commencement of each test, the rat was placed in the box and allowed about 20 min for adaptation. The light was then activated. The time interval between the application of the light beam and a brisk hind-paw withdrawal response was measured. To avoid tissue injury, the cut off time in the absence of a response was 20 sec.

Behavioural and motor function test

The general behaviour (including agitation, allodynia and catalepsy), motor function, pinna reflex, and corneal reflex were examined. The reflexes were judged as present or absent. Agitation was judged to be present when the rat was spontaneously vocalizing or restless. The presence of allodynia was examined by looking for agitation (escape or vocalization) evoked by lightly stroking the flank of the rat with a pencil. Catalepsy was tested by placing the fore-paw slightly higher than the hind-paw. Motor function was evaluated by the placing/stepping reflex and the righting reflex. The former was evoked by drawing the dorsum of either hind-paw across the edge of the table. The latter was assessed by placing the rat horizontally with its back on the table, which normally gives rise to an immediate, coordinated twisting of the body to an upright position.

Experimental paradigm

To determine the time course of analgesic action, the hot-box test, behavioural and motor function tests were performed before and at intervals (15, 30, 45, 60, 90, 120, 180, 240, and 300 min) after epidural or intrathecal morphine injection. For each dose seven epidural to 10 (intrathecal) rats were used.

Data analysis and statistics

Data were expressed as mean ± SEM. Response latency data from hot-box measurements were converted to %MPE according to the formula : %MPE = [(post-drug latency - base-line latency) / (cut off time - base line latency)] × 100. The ED₅₀ values were determined as 50%MPE dose using a locally produced computer programme.

Statistical analysis was performed using two way repeated measures analysis of variance followed by the Contrasts as a post hoc test. $P < 0.05$ was considered statistically significant.

Results

Body weight

Epidural catheterisation did not result in any decrease in body weight while rats with intrathecal catheters lost weight one day after surgery ($P = 0.027$ compared with control, Figure 1).

Effect of volume and dose of epidural morphine

Dose dependent analgesic effects were seen with volumes of 10 and 20 µl but not with 5 µl. The maximum effects were obtained with 20 µl (Figure 2).

FIGURE 1 Body weight.

Bars indicate standard error (SEM). Each group includes 20 rats. *: $P < 0.05$ vs control value (day 0), †: $P < 0.05$ vs the intrathecal group.

FIGURE 2 Effects of volume and dose of epidural morphine. Closed circles indicate 5 µl, open circles 10 µl, and closed triangles show 20 µl. Upper figure is of morphine 30 µg, middle is of morphine 100 µg, and lower is of morphine 300 µg. Mean $\pm$ SEM. Each group includes 7 rats.

*: $P < 0.05$ vs the control value (time 0), †: $P < 0.01$ vs the control value (time 0), ‡: $P < 0.05$ vs 5 µl, §: $P < 0.01$ vs 5 µl, ¶: $P < 0.05$ vs 10 µl.

FIGURE 3 Comparison of the analgesic effect between epidural and intrathecal morphine.

Ep: epidural administration, IT: intrathecal administration. Bars indicate standard error (SEM). Epi-saline and IT-30 µg groups include 10 rats and other groups include 7 rats.

*: $P < 0.05$ vs the control value (time 0), †: $P < 0.01$ vs the control value (time 0), ‡: $P < 0.05$ vs saline, §: $P < 0.01$ vs saline.

Comparison of the analgesic effects between epidural and intrathecal morphine

The effect-time curve of epidural morphine, 300 µg per 20 µl, showed almost the same pattern as intrathecal morphine 30 µg per 10 µl, even though the peak effect was higher and the effect lasted longer after epidural than intrathecal morphine (Figure 3).

The dose-response curve of epidural administration was shifted to the right compared with intrathecal administration (Figure 4).

The ED₅₀ of epidural morphine (94.1 µg, 95% confidence interval; 57.4 to 154.3 µg) was 30 times more than that of intrathecal morphine (3.1 µg, 95% confidence interval; 1.4 to 5.2 µg) ($P < 0.001$).

Behaviour and motor function

Intrathecal morphine 30 µg induced agitation and allodynia in 40% rats, loss of pinna reflex in 20% rats, and motor disturbance in 20% rats, whereas epidural morphine 300 µg showed no side effects (Figure 5).

Discussion

A new rat model of chronic epidural catheterisation has been developed without holes, grooves, or dissection of the bone, and without using glue. This model can provide a clinical relevant analgesic potency ratio of intrathecal:epidural morphine (30:1) when the drug was administered in a volume of 20 µl.

FIGURE 4 Dose-response curves of epidural and intrathecal morphine.

IT: intrathecal administration, Epi: epidural administration. Bars indicate standard error (SEM). IT groups include 10 rats and Epi groups include 7 rats. Horizontal axis is a log scale. *: $P < 0.01$ vs the epidural group.

The first animal model of chronic epidural catheterisation was described by Van den Hoogen *et al.*¹ They used a PE-10 catheter (outer diameter of 0.61 mm), which is larger than the PE-5 catheter (outer diameter of 0.14 mm) we used in this study. The bigger catheter might induce a greater inflammatory reaction than the smaller catheter. Fibrosis around the catheter⁴ might be initiated by an inflammatory reaction. In addition, we did not injure any bone, while in previous studies,^{1,3,4} the investigators made a hole or groove in the bone, or dissected bone. Bone injury might induce an inflammatory reaction and delay recovery from surgery. The use of glue to fix the catheter⁵ also might induce inflammation and tissue injury. The knot that we used was sufficient to fix the catheter without glue. This method might cause less inflammation and is enough to prevent a catheter from being pulled out. We believe that this model is the least invasive in a rat model of chronic epidural catheterisation.

However, in a preliminary study, even in such a less invasive model, fibrosis began two days after catheterisation and occluded the catheter tip four days after catheterisation (Nishiyama *et al.* unpublished data, 1998). Yet, two days after epidural catheterisation, rats recovered completely and analgesic effects did not decrease, while for intrathecal catheterisation, it took five to seven days for recovery. Thus, we could use rats two days after epidural catheterisation, and five to seven

FIGURE 5 Side effects of epidural and intrathecal morphine.

IT: intrathecal administration, Epi: epidural administration, AgAl: agitation and/or allodynia, Pinna: loss of pinna reflex, Motor: motor disturbance. Vertical axis indicates the % of rats which showed each side effect. Total number of rats is 10 in IT saline and IT 30 µg and 7 in other groups.

days after intrathecal catheterisation.

The potency ratio between epidural and intrathecal morphine was 3 judged as assessed from the ED_{50} in the present study. Other experimental studies found ED_{50} values for epidural morphine of 1.5 µg⁵ or 2.2 µg⁴ using the tail flick test, which were similar to those obtained by intrathecal morphine administration (1.2 µg)¹³ using the same test and to that in the present study using the hot-box test (3 µg). Clinically, an adequate dose of intrathecally administered morphine should be 0.2 to 0.4 mg.^{6,7} Usually 3 to 5 mg morphine are administered epidurally for analgesia in humans.⁸⁻¹⁰ Thus, the potency ratio between epidural and intrathecal morphine is about 10 to 30 clinically which is similar to that found in the present study. There might be two reasons for the discrepancy between previous reports and the present results regarding the potency of epidural morphine. First, the drug did not enter the epidural space sufficiently in previous studies¹⁻⁵ because of fibrosis. Second, there may be a difference in the direction of the catheter tip. The catheter was inserted rostrally into lumbar or caudal epidural space by many investigators,¹⁻⁴ while we inserted the catheter caudally into lumbar epidural space. The opposite direction might spread the drug differently. However, the direction of the catheter tip is not as important for the effect of the drug because Kaneko *et al.*⁵ inserted the catheter rostrally into the lumbar space but could not get a greater potency of epidural morphine.

Epidural drug administration has some pharmacodynamic differences from intrathecal injection. Epidurally administered drugs enter the spinal cord by three different routes; directly across the dura, through the perineurium of the nerve roots, or by uptake into arteries or veins. The present study can not determine the principal route in this model. Further pharmacokinetic studies may be useful in elucidating the pathway.

Regarding the optimal volume required to produce hind-paw analgesia; 10 and 20 μ l induced dose dependent effects while 5 μ l did not: 20 μ l induced the maximum effect. Many studies used 20 μ l,^{3,14,15} while some investigators administered 40 μ l⁵ or 100 μ l¹⁶ without any experimental evidence to show optimal effects. A larger volume might have a higher risk of the leakage. Thus, we think that 20 μ l is adequate to administer epidural morphine in the rats.

No side effects were observed using up to 300 μ g morphine by epidural administration although 30 μ g intrathecal morphine induced some side effects. Unfortunately, a dose of more than 300 μ g of morphine could not be tested because 300 μ g is the most that can be dissolved in 20 μ l saline. However, clinically, epidural drug administration induces fewer side effects than by the intrathecal route.

In conclusion, this rat model of epidural catheterisation without bone injury mimics clinical administration of morphine in terms of the comparison with intrathecal administration when the drug was administered in a volume of 20 μ l.

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