

NEUROSCIENCES AND NEUROANAESTHESIA

Chronic intrathecal infusion of gabapentin prevents nerve ligation-induced pain in rats

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Editor's key points

- A rat model of spinal nerve ligation was used to test the efficacy of gabapentin against neuropathic pain.
- Intrathecal gabapentin infusion prevented various pain behaviours, suggesting that neuropathic pain was prevented.

Background. Gabapentin is an anticonvulsant and adjuvant analgesic. It is effective in several pain studies. Neuropathic pain is the most difficult type of pain to treat. In this study, we examined if intrathecal gabapentin could prevent nerve injury-induced pain.

Methods. Under isoflurane anaesthesia, male Sprague–Dawley rats (200–250 g) underwent right L5/6 spinal nerve ligation and placement of an intrathecal catheter connected to an infusion pump. After surgery, intrathecal saline or gabapentin (20 $\mu\text{g h}^{-1}$) was given for 7 days ($n=8$ per group). The right hind paw withdrawal threshold to von Frey filament stimuli and withdrawal latency to radiant heat were determined before (baseline) and once daily for 7 days after surgery. Haematoxylin and eosin and toluidine blue staining were used to evaluate the neurotoxicity of gabapentin (40 $\mu\text{g h}^{-1}$).

Results. Seven days after nerve ligation, the affected paw withdrawal threshold and latency of saline-treated rats decreased from the baseline 11.7 (11.7–22.2) [median (interquartile range)] to 1.6 (0.9–3.2) g and 10.8 (10.5–11.2) to 4.3 (4.2–7) s, respectively. Rats receiving gabapentin (20 $\mu\text{g h}^{-1}$) had higher withdrawal threshold [9.9 (9.9–19.3) g] and latency [11.5 (9.7–11.9) s] on day 7 after ligation. No obvious histopathological change or growth retardation was detected after intrathecal gabapentin (40 $\mu\text{g h}^{-1}$) infusion.

Conclusions. We showed a preventative effect of intrathecal gabapentin on the development of nerve injury-induced mechanical allodynia and thermal hyperalgesia. Our data suggest that continuous intrathecal gabapentin may be considered as an alternative for the prevention of nerve injury-induced pain.

Keywords: gabapentin; intrathecal; neuropathic pain; neurotoxicity; spinal cord, rat

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Gabapentin, 1-(aminomethyl)cyclohexaneacetic acid (Neurontin)[®], was originally developed as a chemical analogue of γ -aminobutyric acid to reduce the spinal reflex for the treatment of spasticity and was found to have anticonvulsant activity.¹ It was later reported to possess analgesic effects in several clinical and animal pain studies, including postoperative,² inflammatory,³ and neuropathic⁴ pain. Unlike morphine, repeated administration of gabapentin does not lead to induction of tolerance,⁵ and gabapentin can attenuate opioid-induced tolerance.⁶ These reports suggest that gabapentin may be an ideal adjuvant to spinal opioid therapy for clinical pain management and suitable for long-term use.

Neuropathic pain can occur as a result of injury or disease to the nerve tissue itself. It is the most difficult type of pain to

treat and is usually refractory to opioid and non-steroidal anti-inflammatory drug treatment.⁷ Many studies have explored the possible mechanisms and potential therapies for the management of such pain. Although animal and clinical studies support the use of gabapentin in the treatment of neuropathic pain, there is no study investigating the effect of continuous intrathecal gabapentin on the development of nerve injury-induced neuropathic pain. Furthermore, no information on the neurotoxicity of intrathecal gabapentin has been reported.

L5/6 spinal nerve ligation provides a classical model of nerve injury pain, producing typical neuropathic behaviour such as mechanical allodynia (pain evoked by a normally non-noxious stimulus) and thermal hyperalgesia (increased pain response to a noxious stimulus) in animals.⁸ In this

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study, using the L5/6 spinal nerve ligation pain model, we determined if continuous intrathecal infusion of gabapentin could prevent nerve injury-induced neuropathic pain. In addition, the possible neurotoxicity of continuous intrathecal gabapentin was investigated.

Methods

The following investigations were performed according to a protocol approved by the Institutional Animal Care Committee of Mackay Memorial Hospital.

Animals

Male Sprague–Dawley rats weighing 200–250 g were housed individually in plastic cages with soft bedding at 22°C in a 12 h light/12 h dark cycle with free access to food and water.

Surgical procedures

All the surgical procedures were performed under inhalation anaesthesia with isoflurane in 100% oxygen, with induction at 5% and maintenance at 2%. Adequate anaesthesia was ascertained by the lack of the ocular reflex and by the absence of a pedal withdrawal response to a hard pinch. During surgery, the percentage of isoflurane was increased, if inadequate anaesthesia was noted. In this study, nine rats showing neurological deficits after surgery were killed with deep isoflurane anaesthesia and intraperitoneal pentobarbital.

L5/6 spinal nerve ligation

Neuropathic pain was induced as previously described.⁹ Rats were anaesthetized and placed prone under a microsurgical apparatus. A midline incision was made and the right paraspinal muscles were separated from the spinous processes at L4–S2. The L5 transverse process was removed and the L4–5 spinal nerves were identified. The nerves were separated and the L5 nerve was tightly ligated with silk thread. The right L6 spinal nerve was then located caudal and medial to the sacroiliac junction and again ligated. For sham surgery, the right L5 and L6 nerves were exposed but not tied. Immediately after surgery, intrathecal catheterization and infusion pump implantation were performed.

Intrathecal catheterization and implantation of infusion pump

Intrathecal catheters were inserted during anaesthesia by passing a PE-5 catheter (filled with normal saline or gabapentin) through an incision in the atlanto-occipital membrane to a position 8 cm caudal to the cisterna at the level of lumbar enlargement. For continuous intrathecal drug administration, an infusion pump (Model 2001, ALZET, Cupertino, CA, USA) with a flow rate of 1 $\mu\text{l h}^{-1}$ was filled with normal saline or gabapentin (20 $\mu\text{g } \mu\text{l}^{-1}$) ($n=8$ for each group) and connected to the catheter. The pump was implanted subcutaneously and the wound was closed. For the long-term follow-up study of 14 days, the implanted pump was removed under isoflurane anaesthesia after behavioural assessments on 7 days after nerve ligation.

Drug

Gabapentin (molecular weight 117.24) was purchased from Sigma-Aldrich (St Louis, MO, USA) and dissolved in normal saline. The infusion dose of 20 $\mu\text{g h}^{-1}$ was chosen according to our previous study.¹⁰

Behavioural assessments

Behavioural tests were performed before and at 7 or 14 days after surgery between 9 a.m. and 3 p.m. by an observer blinded to the treatment groups. Motor function was evaluated by the performance of two specific behavioural tasks: placing/stepping reflex and righting reflex, as previously reported.¹¹

von Frey filament test

The right hind paw withdrawal threshold in response to a normally innocuous mechanical stimuli was determined using von Frey filaments and the up-down method.⁹ Each rat was placed in a transparent plastic dome with a metal mesh floor allowing access to the plantar surface of the right hind paw, and was allowed to acclimatize to this environment for 30 min. The von Frey filament was pressed perpendicular to the plantar surface of the paw with enough force to cause slight buckling of the filament, for about 6 s. A positive response was recorded if the hind paw was sharply withdrawn, or if the animal flinched immediately on removal of the filament.

Plantar thermal test

The latency of right hind paw withdrawal to noxious heat stimuli was measured using the Analgesia Meter apparatus (IITC/Life Science Instruments, Woodland Hills, CA, USA). Rats were placed separately on a temperature-controlled, 3 mm thick glass floor with a light box underneath. They were allowed to become accustomed to the environment for ~30 min before testing. The radiant heat source beneath the glass floor was focused on the plantar surface of the right hind paw. Withdrawal latencies were measured automatically using a photocell. A cut-off time was set at 20 s to avoid damage to the foot. The light intensity was preset to obtain a baseline latency of ~10 s. The withdrawal latencies were collected with at least 5 min intervals, and the middle six of the 10 latencies were averaged.

Rota-rod test

To assess the motor coordination function, rats were placed on a rota-rod (ENV-576, Med. Associates Inc., Georgia, VT, USA), while it was turning at an accelerating speed of 2–20 rpm per 5 min. The retention time on the rod was recorded in seconds, up to a maximum of 300 s during two trials per day for seven consecutive days. Body weight was also measured once daily during the infusion period.

Haematoxylin and eosin staining

To examine the neurotoxicity of intrathecal gabapentin, sham- and nerve-ligated rats received an intrathecal infusion

of saline ($1 \mu\text{l h}^{-1}$) or gabapentin ($40 \mu\text{g h}^{-1}$) for 7 days ($n=6$ in each group). After the infusion, rats were anaesthetized and 4% paraformaldehyde was infused into the heart. The lumbar enlargement of the spinal cord was removed and fixed in 10% formaldehyde. Transverse sections of lumbar enlargement were embedded in paraffin, sectioned ($6 \mu\text{m}$), and stained with haematoxylin and eosin for examination under light microscopy. Histopathological changes were evaluated in a blinded fashion by a senior pathologist (T.-Y.W.) according to a scoring system described previously:¹² 0, absence of abnormal cells; 1, presence of haemorrhage and glial cell reaction in several areas; 2, presence of prominent necrosis in the gray matter, widespread haemorrhage or demyelination, fibrosis, and inflammatory cells.

Toluidine blue staining

Toluidine blue staining was performed to evaluate any neurotoxic effect of intrathecal gabapentin on the cauda equina. After receiving an intrathecal infusion of saline ($1 \mu\text{l h}^{-1}$) or gabapentin ($40 \mu\text{g h}^{-1}$) for 7 days ($n=6$ in each group), sham- and nerve-ligated rats were anaesthetized and the hearts were perfused with phosphate-buffered 2% paraformaldehyde-2.5% glutaraldehyde fixative. The nerve roots caudal to the conus medullaris were dissected and immersed in the same fixative for 4 h, post-fixed in cacodylate-buffered 1% osmium tetroxide, dehydrated, and embedded in epoxy resin. Transverse sections ($1 \mu\text{m}$) were stained with toluidine blue. Neuropathological examination was conducted using light microscopy by a senior pathologist (T.-Y.W.) in a blinded fashion. Quantitative analysis was performed by assigning an injury score to each fascicle present in the cross-section as follows: 0, (no oedema; no injured nerve fibres); 1, mild (mild oedema; little or no nerve fibre degeneration or demyelination); 2, moderate (<50% of nerve fibres with degeneration and demyelination); and 3, severe (more than 50% of nerve fibres with degeneration and demyelination), as described previously.¹³ The injury score for each cross-section was then calculated as the average score of all the fascicles present in the cross-section.

Statistical analysis

Data are presented as median [inter-quartile range (IQR)]. The Mann-Whitney test was used to compare experimental groups. A P -value of <0.05 was considered significant.

Results

Gabapentin blocked the development of nerve ligation-induced mechanical allodynia

Figure 1 shows the temporal changes of right hind paw withdrawal threshold to von Frey filaments during the 7 day infusion period. There was no significant difference pre-surgery (baseline) withdrawal thresholds between the test groups. In rats undergoing nerve ligation with saline infusion, the baseline withdrawal threshold decreased progressively after nerve ligation. Withdrawal thresholds did not change in

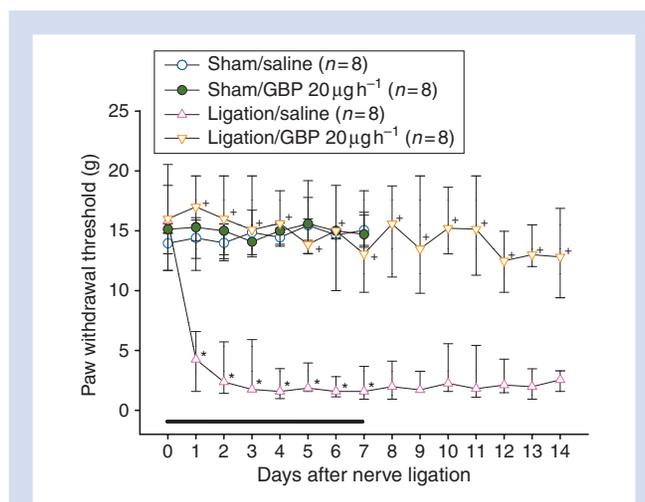


Fig 1 Effect of continuous intrathecal infusion of gabapentin on L5/6 spinal nerve ligation-induced mechanical allodynia, measured as paw withdrawal threshold to von Frey filament stimulus. Bar above the x-axis represents intrathecal infusion with saline ($1 \mu\text{l h}^{-1}$) or gabapentin ($20 \mu\text{g h}^{-1}$) for 7 days. Data are presented as median and IQR. * $P<0.05$ vs the sham/saline group; + $P<0.05$ vs the ligation/saline group (Mann-Whitney test, $n=8$).

rats undergoing sham operation then saline infusion ($P<0.001$). The baseline withdrawal thresholds of sham-operated and ligated rats were similar. Intrathecal infusion of gabapentin $20 \mu\text{g h}^{-1}$ did not increase the paw withdrawal thresholds of sham-operated rats, nor induce motor weakness, as revealed by the normal righting/stepping reflexes and ambulation. During the infusion period, the withdrawal thresholds of ligated rats treated with gabapentin were significantly different from ligated rats receiving saline infusion ($P<0.001$). We also assessed neuropathic behaviour daily up to 14 days after nerve ligation, that is, 7 days after removal of the implanted pump. On post-surgery day 14, the withdrawal threshold of ligated, gabapentin-treated rats significantly differ from ligated rats treated with saline ($P<0.001$, Fig. 1).

Gabapentin blocked the development of nerve ligation-induced thermal hyperalgesia

Figure 2 shows the temporal changes of the right hind paw withdrawal latency to radiant heat stimuli. No significant difference between the groups was found for pre-surgery (baseline) withdrawal latencies. In rats undergoing ligation then saline infusion, baseline withdrawal latency decreased progressively after nerve ligation, whereas there was no change in withdrawal latency of sham-operated animals ($P<0.001$). Baseline withdrawal latencies of sham-operated and ligated rats treated with gabapentin were similar. Intrathecal infusion of gabapentin did not increase the paw withdrawal latencies of sham-operated rats. During the infusion period, the withdrawal latencies of ligated, gabapentin-treated rats were significantly different from those of ligated

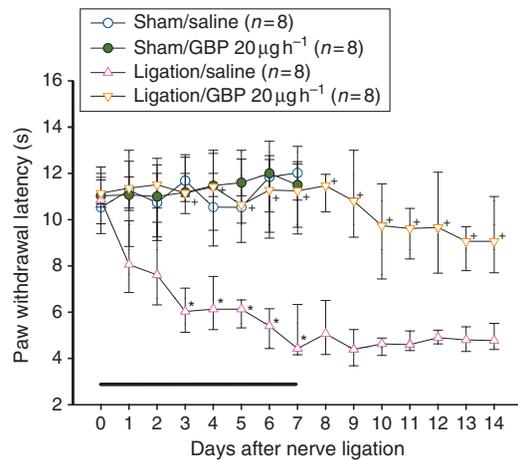


Fig 2 Effect of continuous intrathecal infusion of gabapentin on L5/6 spinal nerve ligation-induced thermal hyperalgesia measured as paw withdrawal latency to radiant heat stimulus. Bar above the x-axis represents intrathecal infusion with saline ($1 \mu\text{l h}^{-1}$) or gabapentin ($20 \mu\text{g h}^{-1}$) for 7 days. Data are presented as the median and IQR. * $P < 0.05$ vs the sham/saline group; + $P < 0.05$ vs the ligation/saline group (Mann-Whitney test, $n = 8$).

animals treated with saline, on days 3–7 (Fig. 2). On post-surgery day 7, the withdrawal latency of ligated/gabapentin-treated animals was significantly different from that of ligated/saline-treated rats ($P < 0.001$).

We also assessed the effect of gabapentin 7 days after infusion had ended. On post-surgery day 14, the withdrawal latency of ligated/gabapentin-treated rats was significantly different from that of ligated/saline-treated animals ($P < 0.001$, Fig. 2).

Higher dose of gabapentin impaired motor coordination without affecting body weight increase

To further investigate the possible neurotoxic effect of intrathecal gabapentin, the infusion dose was increased from 20 to $40 \mu\text{g h}^{-1}$ in sham-operated rats. During the 7 day infusion period, those rats receiving $40 \mu\text{g h}^{-1}$, but not $20 \mu\text{g h}^{-1}$, gabapentin had a significant impairment in their ability to remain on a rotating rod when compared with rats receiving saline infusion (Fig. 3A). The impairment of motor co-ordination seemed not to affect the food-seeking activity of rats, as there was no significant difference in body weight between either dose of gabapentin-treated and saline-treated rats (Fig. 3B).

Higher dose of gabapentin induced no obvious histopathological change in the spinal cord and cauda equina

Figure 4 shows the haematoxylin and eosin-stained lumbar spinal cord sections from sham- and nerve-ligated rats receiving saline or gabapentin ($40 \mu\text{g h}^{-1}$) for 7 days.

Spinal sections had normal histological appearance. Under microscopic examination, no gliosis, demyelination, fibrosis, inflammation, haemorrhage, or necrosis was found at the lumbar level of spinal cords. All animals scored zero.

There was no obvious damage in the fascicles of the cauda equina of either sham- or nerve-ligated rats, whether treated with saline or gabapentin ($40 \mu\text{g h}^{-1}$) for 7 days. Representative fascicles from animals in each group are shown in Figure 5. The injury scores in sham-operated and ligated rats treated with $40 \mu\text{g h}^{-1}$ gabapentin were similar to saline-treated animals ($P = 0.69$ and 0.31 , respectively).

Discussion

The L5/6 spinal nerve ligation model is a classic nerve injury-induced neuropathic pain model developed by Kim and Chung⁸ and produces typical neuropathic behaviours such as mechanical allodynia and thermal hyperalgesia within days of nerve ligation. In our study, using this model, we found that intrathecal infusion of gabapentin ($20 \mu\text{g h}^{-1}$) for 7 days prevented nerve ligation-induced pain and the effect was sustained for 7 days after drug discontinuation. At a higher dose ($40 \mu\text{g h}^{-1}$), gabapentin induced no growth retardation or obvious neuropathological changes in the spinal cord and cauda equina. Our findings suggest that continuous intrathecal gabapentin may be considered for the prevention of nerve injury-induced pain. To our knowledge, this is the first study investigating the effect of chronic intrathecal infusion of gabapentin on the development of nerve injury-induced neuropathic pain.

Gabapentin has been found to be effective in many types of pain, including post-herpetic neuralgia, trigeminal neuralgia, and nerve ligation-induced neuropathic pain.^{14–15} In addition, gabapentin has been reported to suppress spasticity in spinal cord-injured rats and act synergistically with intrathecal clonidine in postoperative and nerve ligation pain models.^{2–16–17} The possible mechanisms involved in gabapentin action include binding to the $\alpha 2\delta$ subunit of Ca^{2+} channels,^{18–19} which are important for neurotransmitter release, or inhibiting Na^+ currents.²⁰ Gabapentin has been reported to reduce glutamate release from the spinal dorsal horn in neuropathic and inflamed rats.²¹ Through binding to pre-synaptic $\alpha 2\delta$ subunit of Ca^{2+} channels, gabapentin might be able to reduce spinal glutamate release to achieve its preventive effect in this study. It is interesting to note that $\alpha 2\delta$ -1 subunit was recently identified to be a receptor involved in excitatory synapse formation and gabapentin may act by blocking new synapse formation.²² Gabapentin has also been found to inhibit spinal microglial activation in streptozotocin-induced diabetic rats.²³ Microglial activation is well known to play an important role in the development of nerve injury-induced pain²⁴ and glutamate could induce chemotactic responses of microglial cells.²⁵ It is possible that microglial inhibition may also be involved in the action of gabapentin observed in this study.

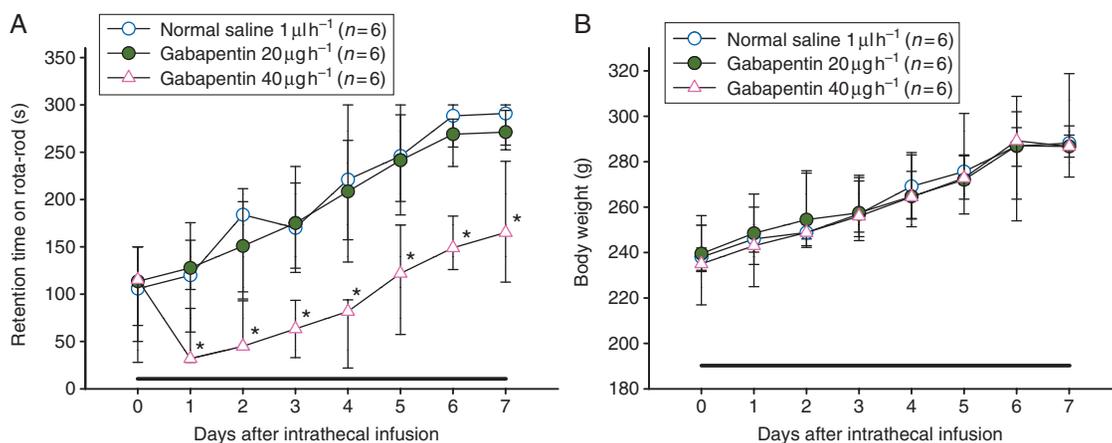


Fig 3 Effects of intrathecal gabapentin infusion on motor co-ordination, measured as time spent on accelerating rota-rod (A) and body weight changes (B). Bar above the x-axis represents intrathecal treatment with saline or gabapentin. Data are presented as median and IQR. * $P < 0.05$ vs the saline group (Mann–Whitney test, $n = 6$).

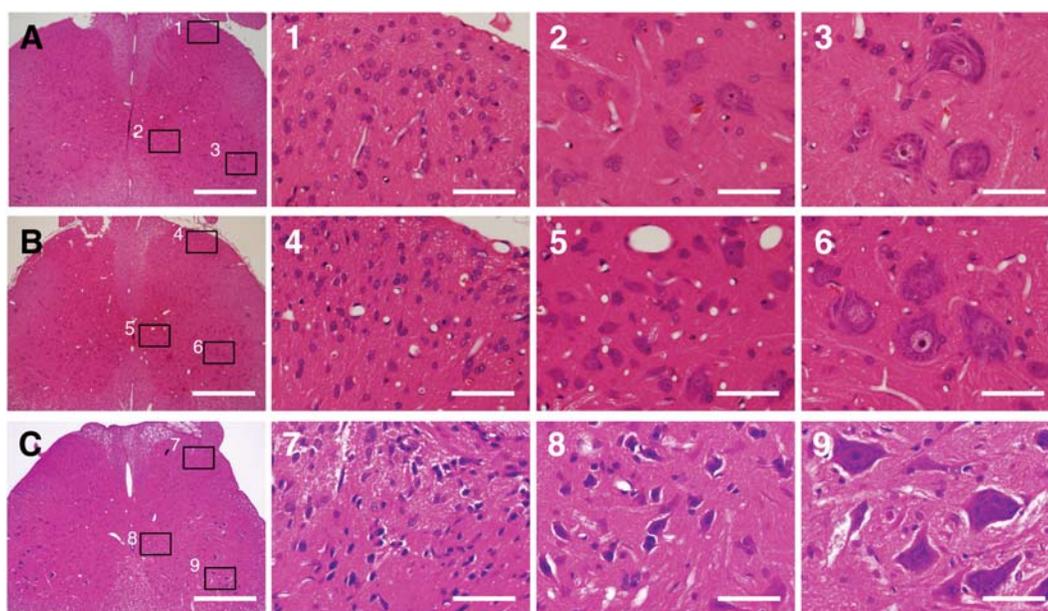


Fig 4 Representative photomicrograph showing haematoxylin and eosin staining of lumbar spinal cord sections from sham-operated (A and B) and nerve-ligated (C) rats after receiving intrathecal infusion of normal saline $1 \mu\text{l h}^{-1}$ (A) or gabapentin $40 \mu\text{g h}^{-1}$ (B and C) for 7 days. All rats were scored at zero ($n = 6$). 1–9, Higher magnification images of dorsal, medial, and ventral insets in A–C, respectively. Scale bar: $500 \mu\text{m}$ (A–C), $50 \mu\text{m}$ (1–9).

For a drug to be tested intrathecally in clinical trials, it is imperative to examine its neurotoxic effects first in animals. For instance, intrathecal lidocaine has been found to induce neuropathological changes in the spinal cord and cauda equina.²⁶ Intrathecal gabapentin has been reported to induce motor weakness in rats after a large dose.²⁷ In this study, $20 \mu\text{g h}^{-1}$ of intrathecal gabapentin produced an almost maximal effect in terms of preventing nerve ligation-induced pain. We therefore doubled the dose and

found that a higher infused dose ($40 \mu\text{g h}^{-1}$) impaired the motor co-ordination of rats, as evidenced by the performance on the rota-rod. However, this dysfunction did not prevent the rats from normal weight gain, indicating that this side-effect is not severe enough to interfere with their food-seeking activity. Cho and colleagues²⁸ have also reported that intrathecal gabapentin, at 100 and $300 \mu\text{g}$, inhibited rat motor coordination. In our study, no obvious histopathological change in the spinal cord and cauda equina

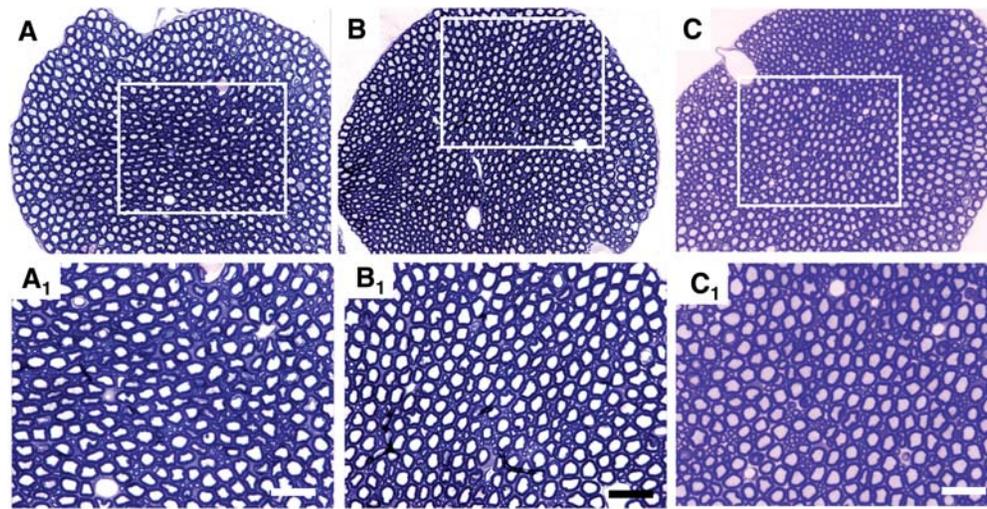


Fig 5 Representative photomicrograph showing toluidine blue staining of the cauda equina fascicle sections from sham-operated (A and B) and nerve-ligated (C) rats after receiving intrathecal infusion of saline $1 \mu\text{l h}^{-1}$ (A) or gabapentin $40 \mu\text{g h}^{-1}$ (B and C) for 7 days. (A₁–C₁) Higher magnification images of the insets in (A–C), respectively. Injury scores of the three groups were not significantly different ($n=6$). Scale bar: $20 \mu\text{m}$.

was found after chronic intrathecal infusion of $40 \mu\text{g h}^{-1}$ gabapentin. However, more detailed neurotoxicity studies, including the doses tested in this study, should be performed in the future before intrathecal gabapentin is considered for clinical use.²⁹ Oral intake of gabapentin has been reported to induce mild-to-severe side-effects in patients, such as ataxia, nystagmus, myopathy, renal failure, and encephalopathy.^{30 31} In this regard, intrathecal infusion of gabapentin might be considered as an alternative route of administration to avoid these systemic side-effects, especially for patients with complex pain syndromes.³²

Intrathecal morphine has been used to treat intractable cancer pain. However, its use has been associated with analgesic tolerance and severe side-effects.³³ Intrathecal gabapentin has been reported to enhance the analgesic effects of morphine in a rat pancreatitis model³⁴ and attenuate spinal morphine-induced tolerance.⁶ Recently, gabapentin was shown to act synergistically with morphine and clonidine in neuropathic pain models.^{17 35} Animal studies also revealed a neuropathic component in the pathogenesis of cancer pain.³⁶ Taken together with our findings, it is suggested that gabapentin may be co-administered intrathecally with morphine or clonidine to enhance their analgesic effects and prevent morphine-induced tolerance in cancer pain patients.^{32 37}

Conclusions

The present study demonstrates that continuous intrathecal infusion of gabapentin prevents the development of nerve ligation-induced mechanical allodynia and thermal hyperalgesia in rats. At the dose tested, no obvious neurotoxicity was observed. To our knowledge, this is the first study examining the effect of continuous intrathecal gabapentin in the

spinal nerve ligation pain model. Our results suggest that intrathecal gabapentin infusion may provide a new strategy for the prevention of nerve injury-induced neuropathic pain.

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