

Cytidine 5'-diphosphocholine administration prevents peripheral neuropathic pain after sciatic nerve crush injury in rats

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Background: Cytidine 5'-diphosphocholine (citicoline) has been shown to have beneficial effects in central nervous system injury as well as in motoric functional recovery after peripheral nerve injury. This study aimed to examine the effect of citicoline on prevention of neuropathic pain in a rat model of sciatic nerve crush injury.

Methods: Forty experimental rats were divided into four groups. In three groups, the right sciatic nerves were crushed in the mid-thigh region, and a gelatin sponge moistened with 0.4 or 0.8 mL of 100 $\mu\text{mol/L}$ citicoline, or saline 0.4 mL in the control group, was applied. The fourth group of rats was sham-operated, ie the sciatic nerve was exposed with no crush. Functional assessments were performed 4 weeks after crush injury. von Frey filaments (100 g threshold) were used to assess neuropathic pain. In addition, the sciatic functional index and extensor postural thrust (EPT) tests were used to assess motoric function.

Results: The crush/citicoline 0.4 mL group had a lower percentage of pain (23.53%, $n=17$) compared with the crush/saline group (53.33%, $n=15$, $P<0.005$). The crush/citicoline 0.4 mL group also showed better motoric recovery, as seen in stronger EPT results ($P<0.001$). However, the sciatic functional index analysis did not show significant differences between groups ($P=0.35$). The crush/citicoline 0.8 mL group showed a higher percentage of pain (66.67%, $n=18$) and less EPT recovery. These results may be explained by more severe nerve injury due to compression with a larger administered volume.

Conclusion: In situ administration of 0.4 mL of 100 $\mu\text{mol/L}$ citicoline prevents the occurrence of neuropathic pain and induces motoric recovery, evaluated by EPT test, 4 weeks after sciatic nerve injury.

Keywords: nerve injury, nerve regeneration, neuropathic pain

Introduction

Neuropathic pain is an issue that has not been thoroughly studied or resolved. It is defined as pain that is caused by a primary lesion in or damage to the central or peripheral nervous system. Damage may occur as a result of compression, cutting, ischemic or metabolic disorders, cellular infiltration, or a combination of the above.¹ If there is damage to the nerves, then there will be a cascade reaction, starting with damage in the nerve cell membranes and progressing to cell edema, Wallerian degeneration of the distal segments, and the cell regeneration process in the form of axonal sprouting. Unsuccessful regeneration may result in neuroma, and neuroma formation after injury is one of the main mechanisms responsible for the onset of neuropathic pain.²

Neuropathic pain affects 2%–3% of the population and is very problematic because of its severity, chronicity, and resistance to treatment, as well as its effects

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on reducing patient quality of life. The costs of neuropathic pain treatment are high, and the success rate with the use of drugs that already exist is less than 50%.^{3,4} The unclear pathophysiology of neuropathic pain is one reason for the difficulty in determining the right medication. Nowadays, doctors are more likely to use drugs that inhibit the excessive transmission of sodium and calcium ions to repress ectopic discharge that arises as a result of nerve damage that becomes the source of pain.

Citicoline has been proven to induce nerve regeneration after injury in several *in vitro* studies.⁵⁻⁷ It has been widely used in clinical cases of central nervous system disorders such as ischemic stroke, cognitive impairments,⁸ and glaucoma.⁹ *In situ* administration of citicoline has been reported to play a role in improving motoric function and inducing the regeneration process of the damaged axons in a rat sciatic nerve injury model.⁵ Administration of CDP-choline and choline + cytidine several days after injury shows improved motoric function recovery and better structural nerve regeneration,⁶ indicating its potential in the treatment of peripheral nerve injury. However, there are no reports about whether administration of citicoline may prevent neuropathic pain after peripheral nerve injury.

Based on the above, this study aimed to investigate the effect of citicoline administration on neuropathic pain in a rat model of injured sciatic nerve. This study will assess regenerative improvement through pain behavior assessment as well as motoric functional analysis.

Materials and methods

Animals

Forty adult male Wistar rats weighing 200–300 g were used in this study. Acclimatization was done at least 1 week before surgery. Groups of four rats were put in plastic cages with padded floors and kept in a laboratory animal care facility with a light/dark cycle of 12/12 hours (light from 8 am to 8 pm and dark from 8 pm to 8 am). Rats were given free access to food and water. This experiment was approved by the Ethical Committee of the Faculty of Medicine of the Universitas Gadjah Mada, Yogyakarta, Indonesia (no KE/FK/478/EC). All procedures that were performed to the mice following the Law of the Republic of Indonesia Number 18/2009 on Husbandry and Animal Health and National Ethical Guidelines for Health Research 2011.

Surgical procedures

Rats were anesthetized by intramuscular injection of 30 mg/kg thiopental sodium. The fur of the gluteal area was shaved

and topical povidone/iodine was applied. Incision was made through the skin and gluteal muscle until the right sciatic nerve was identified. The location to be crushed was marked with 8.0 nylon thread in the epineurium. The nerves were crushed using an arterial clamp on the strength of one tooth for 60 seconds.¹⁰ The forty rats were grouped randomly into a crush/saline control group (n=10), crush/0.4 mL citicoline treatment group (n=10), or crush/0.8 mL citicoline treatment group (n=10). The injured nerves of the groups were then wrapped with a gelatin sponge soaked with 0.4 mL of 0.9% saline solution, 0.4 mL of 100 µmol/L citicoline, or 0.8 mL of 100 µmol/L citicoline, respectively. A further group of rats was sham-operated, ie the right sciatic nerve was exposed without any injury or treatment given (n=10). Antibiotics were given *in situ* and the wounds were closed layer by layer. The whole surgical procedure was done by one operator.

Evaluation of neuropathic pain behavior

von Frey filament tests with a threshold of 100 mg were performed in the fourth week after surgery. Pressure was applied to the third and fourth toes until the legs bent (withdrawal response). The result was considered positive if there was a withdrawal response of seven or more out of ten repetitions.¹¹

Sciatic functional index

Sciatic functional index (SFI) was measured using walking tracks made from plywood (10 cm × 100 cm dimension), which were covered with white paper and led to a dark compartment. Both hind limbs of each rat were dipped in methylene blue stain. Four different measurements of the rat footprints were obtained: distance footprints, spread of fingers, distance between the footsteps, and distance between the legs. SFI was calculated based on the values obtained using the following equation:¹²

$$SFI = \left[\left(\frac{NPL - EPL}{EPL} \right) + \left(\frac{ETS - NTS}{NTS} \right) + \left(\frac{EIT - NIT}{NIT} \right) \right] \frac{220}{3} \quad (1)$$

SFI=Sciatic Funtional Index, N: normal, or non-operated; E: experimental, or operated; PL: print length; TS: total toe spread, or distance between first to fifth toe; IT: intermediate toes spread, or the distance between the second and fourth toes.

Extensor postural thrust (EPT) test

Using the examiner's hand, each rat's body weight was supported at the thoracic area and the right leg was made to touch

to the weighing surface of the scale. When there was contact between the rat's toes and the scale, the rat would straighten its leg and kick the scale. The numbers that appeared on the monitor of the scale were recorded in grams as the strength of the right leg muscles and regarded as experimental EPT (EEPT). The same procedure was applied for the left hind leg (normal EPT [NEPT]). Motor deficit was calculated using the following equation:¹³

$$((NEPT - EEPT) / NEPT) \times 100\% \quad (2)$$

Statistical analysis

The results regarding neuropathic pain behavior were reported as frequency of positive pain behavior for each group and analyzed using the chi-square test. The mean differences between groups for the SFI and EPT were assessed using Kruskal–Wallis statistical analysis. *P*-values <0.05 were considered statistically significant.

Results

Neuropathic pain behavior test

The results of the von Frey filament tests, performed in the fourth postoperative week and using 100 g as the cut-off point, are shown in Table 1. Most of the rats with an injured sciatic nerve that had saline treatment showed pain behavior (8/10), whereas pain behavior was only detected in 2/10 of the injured rats that received 0.4 mL citicoline (*P*<0.05). More rats that were treated with 0.8 cc citicoline showed pain behavior (4/10), but there was no statistically significant difference in comparison with the saline group (*P*>0.05).

SFI

Based on the walking track analysis, SFI values, as indicators of functional recovery, were calculated, and the results are shown in Table 2. The results show that the difference between groups was not statistically significant (*P*=0.26) (Table 2).

EPT

EPT tests were conducted in the fourth week after surgery, and the results are shown in Table 3. As predicted, the

Table 1 Pain behavior assessed with von Frey filaments (100 g threshold) 4 weeks after crush injury

| Group | Neuropathic pain (+), % | χ^2 | <i>P</i> |
|-------------------------|-------------------------|----------|----------|
| Crush/saline | 80 | | |
| Crush/citicoline 0.4 cc | 20 | 5.05 | 0.02 |
| Crush/citicoline 0.8 cc | 40 | 1.82 | 0.18 |
| Sham operation | 0 | 10.77 | 0.001 |

Table 2 SFI 4 weeks after crush injury

| Group ^a | SFI | SD | Min | Max | <i>P</i> |
|-------------------------|-------|-------|------|-------|----------|
| Crush/saline | 25.98 | 17.89 | 0.12 | 48.48 | 0.264 |
| Crush/citicoline 0.4 mL | 19.82 | 22.3 | 0.57 | 69.65 | |
| Crush/citicoline 0.8 mL | 25.45 | 12.58 | 5.32 | 41.61 | |
| Sham operation | 13.65 | 11.02 | 0.17 | 32.02 | |

Notes: ^an=10 rats per group. Kruskal–Wallis statistical analysis.

Abbreviations: SFI, sciatic functional index; SD, standard deviation; Min, minimum; Max, maximum.

sham-operated rats showed a better result (mean 2.47%) compared to the injured groups. Injured rats that had the 0.4 mL citicoline treatment showed the best result among the injured groups (14.28%), with the injured rats that received saline having the worst result (36.54%). The 0.8 mL citicoline administration led to a less satisfactory EPT result (20.60%) compared to the 0.4 mL citicoline group. The results were statistically significant (*P*=0.00).

Discussion

To our knowledge, this is the first report showing the effect of citicoline in preventing pain behavior after peripheral nerve injury. Pain behavior testing showed that the group treated with 0.4 mL citicoline had fewer rats that had neuropathic pain compared to the other injured groups. This suggests that citicoline has the potential to prevent the onset of neuropathic pain based on its effect in inducing optimal axonal regeneration. However, more of the rats which were treated with the higher volume of citicoline (0.8 mL) showed pain behavior and this may have been due to excessive compression of the injured nerve with a gelatin sponge that had absorbed more volume. Another possibility is that the sponge's absorbing capacity was exceeded, causing a spill of citicoline to the surrounding tissue that may have contributed to inducing more inflammation and resulted in inhibition of axonal regeneration.

The study of functional status using walking track analysis showed a nonsignificant result. This was presumably due to

Table 3 Motoric deficit assessed by extensor postural thrust test

| Group | Motoric deficit, % | | | SD | <i>P</i> |
|-------------------------|--------------------|-------|-------|-------|----------|
| | Mean | Min | Max | | |
| Crush/saline | 36.53 | 20 | 53.59 | 12.08 | 0.000 |
| Crush/citicoline 0.4 mL | 14.28 | 3.78 | 26.00 | 8.16 | |
| Crush/citicoline 0.8 mL | 20.6 | 12.48 | 29.39 | 5.53 | |
| Sham operation | 2.47 | 12 | 12.88 | 8.37 | |

Notes: Kruskal–Wallis test. post hoc Mann–Whitney test: crush/saline versus crush/citicoline 0.4 mL, *P*=0.001; crush/saline versus crush/citicoline 0.8 mL, *P*=0.005; crush/saline versus sham operation, *P*<0.001.

Abbreviations: Min, minimum; Max, maximum; SD, standard deviation.

the difficulty in measuring footprint lengths, as the footprints were sometimes not clearly visible. Some animal is sometimes less cooperative when walking the tracks. A previous study mentioned the same constraints in SFI calculation.¹² Further studies may determine methods that are easier to implement and that have more accuracy.

EPT is another motoric functional test that was conducted in this study. The EPT result was in concordance with the result of the pain behavioral test, ie 0.4 mL citicoline administration resulted in the best functional recovery, while the 0.8 mL citicoline group had a less satisfactory result. Our results also support the motoric functional recovery after citicoline administration in sciatic nerve injury reported by Ozay et al.⁵

The mechanism of citicoline in improving axonal regeneration post-injury needs to be clarified. CDP-choline and its metabolites, choline and cytidine, had a beneficial effect in nerve repair after peripheral nerve injury in our rat model. Choline and cytidine whether alone or in combination, are important substrates for the synthesis of phosphatidylcholine and promote cell membrane synthesis and repair,^{14–17} as well as neurogenesis in vitro or in vivo.^{18,19–21} Administered CDP-choline exogenously produces some pharmacologic effects that increase cholinergic neurotransmission.^{22–25} Some studies have proved the effects of choline in decreasing the severity of inflammatory response²⁶ and preventing endotoxin-induced multiorgan injury.^{26,27} All these effects are essential for the recovery process after nerve injury.^{28–30} Since oral and intravenous citicoline administration has shown potential in entering injured nervous tissue in clinical settings, we plan to test in the future whether systemic administration of citicoline may give similar results in preventing neuropathic pain in injured sciatic nerves.

Conclusion

We have shown that the administration of 0.4 mL citicoline in situ can prevent neuropathic pain after sciatic nerve crush injury in rats.

Disclosure

The authors report no conflicts of interest in this work.

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