

Effect of Gabapentin and Baclofen on Histology Study in Neuropathic Pain

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Abstract

Neuropathic pain resulted from injury to nerves is often resistant to current treatments and can seriously cause chronic pain if no appropriate treatment is given. This study was designed to prove the effectiveness of gabapentin and baclofen in increasing latency time toward thermal stimulus and recovering the morphology of dorsal horn of spinal cord in neuropathic-induced chronic pain. Forty mice were divided into 8 groups i.e sham, negative control, gabapentin at three different doses (10, 30, 100 nmol) and baclofen at three different doses (1, 10, 30 nmol). Neuropathic condition was induced by ligation of sciatic nerve with Partial Sciatic Nerve Ligation (PSNL) method. Gabapentin and baclofen were administered intrathecally once a day for seven days, a week after neuropathic induction. Latency time toward thermal stimulus was measured on days 0, 1, 3, 5, 7, 8, 10, 12 and 14 after induction. Histology of the dorsal horn of spinal cord tissue was examined by haematoxylline-eosin staining. The results showed that intrathecal injection of gabapentin and baclofen significantly increased latency time of mice toward thermal stimulus compared with negative control. Gabapentin and baclofen are effective as treatment for neuropathic pain. They can also help the recovery process of the histology in dorsal horn in neuropathic pain.

Keywords: Baclofen, dorsal horn, gabapentin, neuropathic pain, PSNL

Efek Gabapentin dan Baclofen terhadap Studi Histologi pada Nyeri Neuropati

Abstrak

Nyeri neuropati berasal dari luka pada saraf yang umumnya sulit diterapi sehingga menyebabkan nyeri kronik bila tanpa manajemen terapi yang tepat. Tujuan penelitian ini adalah membuktikan efektivitas gabapentin dan baclofen dalam meningkatkan waktu ketahanan terhadap panas dan memperbaiki morfologi *dorsal horn* dari *spinal cord* pada keadaan nyeri kronik yang disebabkan neuropati. Empat puluh mencit terbagi ke dalam delapan kelompok, yaitu *sham*, kontrol negatif, gabapentin (dosis 10, 30 dan 100 nmol) serta baclofen (dosis 1, 10 dan 30 nmol). Nyeri neuropati diinduksi menggunakan metode *Partial Sciatic Nerve Ligation* (PSNL). Gabapentin dan baclofen diberikan secara intratekal satu kali sehari selama tujuh hari pada satu minggu setelah induksi nyeri neuropati. Waktu ketahanan terhadap stimulus panas diamati pada hari ke-0, 1, 3, 5, 7, 8, 10, 12, dan 14 setelah induksi. Histologi *dorsal horn* dari *spinal cord* mencit diamati menggunakan pewarnaan *haematoxylline-eosin*. Injeksi intratekal gabapentin dan baclofen meningkatkan waktu ketahanan terhadap stimulus panas secara signifikan dibandingkan kontrol negatif. Gabapentin dan baclofen efektif sebagai terapi nyeri neuropati. Keduanya juga dapat memperbaiki histologi *dorsal horn* pada kondisi nyeri neuropati.

Kata kunci: Baclofen, *dorsal horn*, gabapentin, nyeri neuropati, PSNL

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Introduction

International association study of pain defines pain as an unpleasant sensory and emotional experience associated with actual or potential damage.¹ Many people think that it is a simple condition. However neglected pain treatment can cause a condition that called chronic pain, especially when it lacks an appropriate treatment. For example neuropathic pain, one of the conditions that lead to chronic pain, is a spontaneous pain and hypersensitivity to pain in association with damage or a lesion of a nervous system. Recent studies show that neuropathic pain incidence increases in every years. In Europe, chronic pain is estimated to 55.2%. Twenty five percent patient with chronic pain or about 1.5 to 3% of the population will have neuropathy.² In Indonesia was reported that 25–50% of elderly population have pain. Until now, pathophysiology of neuropathic pain is still not properly understood, so an appropriate treatment for this condition is still be a challenge.³

Neuropathic pain pathophysiology involves an imbalance between excitatory (such as glutamate) and inhibitory neurotransmitter (such as GABA). NMDA, one of glutamate subunit is the important key in this pathways. NMDA receptor consist of 3 subunit ie (NR) 1, NR2 (A,B,C,D) dan NR3 (A,B).⁴ NR2B is the most important subunit receptor of NMDA that plays essential role in dorsal horn of spinal cord and as a lead protein in tyrosine phosphorylation, an important key for neuropathic pain. Higher NR2B expression was reported in neuropathic condition.⁵ So, by increasing the activation of inhibitory neurotransmitter, GABA will appropriate as an alternative management therapy.

Gabapentin and baclofen are drugs that primarly used as antiepilepsy and antispasticity. Both of them are often used as adjuvant treatment in neuropathic pain.^{6,7}

Nowadays, many researchs are developed to find alternative strategy drugs for neuropathic pain treatment. Latest study shows that Gabapentin and Baclofen have an opportunity for first line treatment in neuropathic pain, replace another drugs such as morphine that have a lot of limitation of use.^{8,9} Gabapentin had the highest levels of evidence when used in patient with spinal cord injury (SCI).¹⁰ Gabapentin and baclofen as known as GABA agonist increase GABA activity with two different mechanisms. Gabapentin binds to $\alpha 2\delta 1$ ubunit in Ca^{2+} canal and inhibits glutamate release.^{9,11} The other side, baclofen is an GABAB agonist receptor that stimulates hiperpolarization by increasing K^+ influx and decreasing Ca^{2+} influx then inhibits release of glutamate.¹² This drugs may have a future role in treatment of neuropathic pain, but no data are available to support this at present. In this research, we try to get an explanation the effectiveness of gabapentin and baclofen as treatment for neuropathic pain and how their action to recover the histology in dorsal horn in neuropathic pain condition.

Methods

Forty of eight weeks males mice were used for the experiment. They were purchased and kept in the animal house, College of Pharmacy, University of Airlangga, Surabaya, Indonesia. The temperature of the room was maintained at 26–28 °C with a 12-hour light/12-hour dark cycle. Gabapentin and Baclofen were purchased from Sigma. Each of them was dissolved in 1 mL of normal saline. Gabapentin and baclofen were administered as a solution intratechally.

Mice were weighed and divided into 8 groups of 5 each : On day 0, each mouse in group 1–7 (neuropathy group) was induced neuropathy with PSNL method. Mice were anesthetized with aether and tying 1/3–1/2 of dorsal portion of sciatic nerve on the

left lumbar nerve of mice with 8–0 silk. In group 8 (Sham group), the sciatic nerve was exposed without ligation. On day 7–13, each group will received a different treatment:

- Group I : normal saline 5 μ L (negative control)
 Group II : gabapentin 10 nmol 5 μ L
 Group III : gabapentin 30 nmol 5 μ L
 Group IV : gabapentin 100 nmol 5 μ L
 Group V : baclofen 1 nmol 5 μ L
 Group VI : baclofen 10 nmol 5 μ L
 Group VII : baclofen 30 nmol 5 μ L
 Group VIII : normal saline 5 μ l (sham)

Behavioral study was measured time latency toward thermal stimulus stimulation with hot plate at temperature 48 °C on day 0, 2, 4, 6, 7, 8, 10, 12, and 14. On day 14th, all mice were sacrificed with decapitation and spinal organ of each mouse was immediately removed. Each spinal cord was fixed in neutral buffer formalin 10% for 24 hours. These samples were then routinely processed using the haematoxylin and eosin staining method for histology study. Samples of dorsal horn of spinal cord were observed with 1000x magnification. Representations of dorsal horn include nucleus performance, inflammatory cell (microglia), another glia cell and vasodilatation of blood vessel.

Time towards thermal stimulus was expressed as mean \pm SEM. The significant differences between neuropathy and sham group on day 0,1,3,5 and 7 were tested by

independent t-test. The significant differences between groups on day 8,10,12, and 14 were tested by one-way ANOVA followed by Tukey's HSD. Differences of p value of less than 0.05 were considered statistically significant. Histology of dorsal horn of spinal cords was explained by a descriptive method.

Result

On day 0, before neuropathic induction, the time latency towards thermal stimulus in neuropathy groups (group 1–7) was similar to it of sham group (group 8). In the other hand, the time latency toward thermal stimulus in neuropathy groups after neuropathic induction (on day 1–7) were lower than sham group. The average of time toward thermal stimulus of each groups on day 0, 1, 3, 5, and 7 are given in Table 1.

After administration of gabapentin and baclofen, the time towards thermal stimulus was higher than before. The average of time toward thermal stimulus of each groups on day 8, 10, 12 and 14 are given in Table 2. Neuropathy induction with PSNL method caused histological changes in morphology of dorsal horn of spinal cord. The histology differences between neuropathy and sham groups are given in Figure 1.

Administration of gabapentin and baclofen repaired histology of dorsal horn of spinal cord from mice. The histological changes with and without drug administration are

Table 1 Average Time toward Thermal Stimulus Between Neuropathy and Sham Grups on day 0, 1, 3, 5, and 7

No	Groups	Average time toward thermal stimulus on day (second)				
		0	1	3	5	7
1	Neuropathy (35 mice)	9.5 (0.2)	5.3 (0.1)*	4.2 (0.1)**	3.9 (0.1)***	3.4 (0.1)****
2	Sham (5 mice)	9.4 (0.4)	7.2 (0.3)	8.6 (0.3)	10.2 (0.7)	11.9 (0.9)

Note: Data are presented as mean (SEM), *)p < 0.001; **)p < 0.01; ***)p < 0.001; ****)p < 0.01 versus Sham group

Table 2 Average Time toward Thermal Stimulus after Administration of Gabapentin and Baclofen on Day 8, 10, 12, and 14

No	Groups	Treatment	Average time toward thermal stimulus on day SEM (second)			
			8	10	12	14
1	Neuropathy	Normal saline	3.5 (0.2)	3.2 (0.2)	3.2 (0.2)	3.2 (0.3)
2		Gabapentin 10 nmol	7.2 (0.4)	8.8 (0.2)	10.4 (0.3)	11.3 (0.5)
3		Gabapentin 30 nmol	8.0 (0.3)	9.1 (0.3)	11.0 (0.5)	11.7 (0.7)
4		Gabapentin 100 nmol	9.3 (0.1)	10.9 (0.3)	12.3 (0.6)	14.0 (0.7)
5		Baclofen 1 nmol	6.8 (0.1)	8.7 (0.4)	9.7 (0.3)	11.1 (0.5)
6		Baclofen 10 nmol	7.8 (0.2)	9.4 (0.4)	11.2 (0.2)	11.7 (0.3)
7		Baclofen 30 nmol	8.8 (0.3)	10.7 (0.4)	11.6 (0.6)	13.3 (0.5)
8	Sham	Normal saline	10.6 (0.3)	10.9 (0.5)	10.9 (0.8)	10.0 (0.3)

given in Figure 2 and 3.

Discussion

Patients who have neuropathic pain perform report that there is a wide variety of unusual sensations or pain complaints. These may include burning pain, pain radiating into an area of anesthesia, or an absent limb, pulling, drawing or crawling sensations, lancinating explosive pains which may be spontaneous in their origin or induced by mechanical or thermal stimulation. Until now, pathophysiology of

neuropathy pain is still poorly understood. In human, neuropathy condition contributes to neuronal damage, particularly at L4 and L5 of the spinal cord. According to Bridges, et al¹³, PSNL is widely used because this method is analogical with human condition and causes 60% hyperalgesia and allodynia.

Neuropathy pain causes hyperalgesia and allodynia that can be seen by increasing the latency time towards thermal stimulus. The damages of nerves cause relief of pain sensitization. Besides, neuropathy pain causes

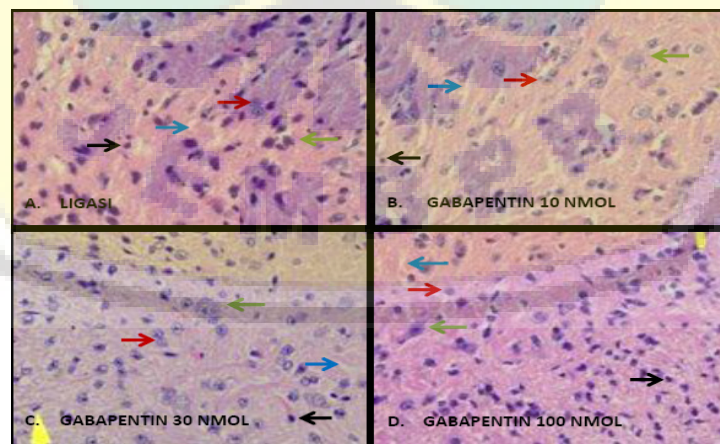


Figure 2 Photomicrograph of the dorsal horn from spinal cord of mice between neuropathy group (A= without treatment) and gabapentin treatment (B= 10 nmol; C= 30 nmol; D= 100 nmol), with haematoxylin and eosin staining (400x magnification). Treatment group showed positive changes in morphology of dorsal horn ie inflammatory cell, number of neuron body and glia cell, and also vasodilation of blood vessel. Red arrow: neuroglia (not microglia), Black arrow: inflammatory cell, Green arrow: neuron body, Blue arrow: blood vessel.

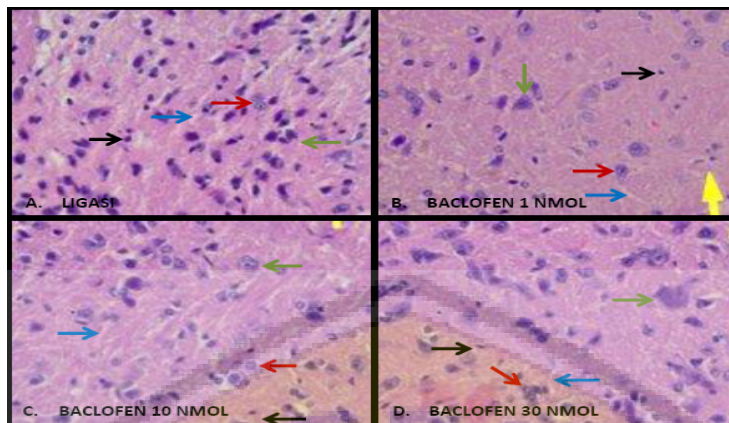


Figure 3 Photomicrograph of the dorsal horn from spinal cord of mice between group neuropathy (A = without treatment) and baclofen treatment (B= 1 nmol; C= 10 nmol; D = 30 nmol), with haematoxylin and eosin staining (400x magnification). Treatment group showed positive changes in morphology of dorsal horn ie inflammatory cell, number of neuron body and glia cell, and also vasodilation of blood vessel. Red arrow : neuroglia (not microglia), Black arrow: inflammatory cell, Green arrow: neuron body, Blue arrow : blood vessel

changing of morphology of dorsal horn in spinal cord such as increasing inflammatory cell (microglia), prominent nucleolus infiltration, and vascular permeability also decreasing neuron body and others glia cell.

Neuropathy causes signal transduction and followed by releasing of inflammatory mediators (prostaglandins, bradykinine, histamine, and serotonin) results activation of nociceptor. This process is followed by pain transmission by A δ and C nerve fibers from dorsal horn of spinal cord to the brain, then induces release of excitatory neurotransmitter (i.e glutamate and substance P). Glutamate binds non NMDA receptors (i.e AMPA and kainite), whereas substance P binds to NK-1 receptors, cause depolarization. Continuous depolarization causes inhibition loss of Mg²⁺ blockade on NMDA receptor channel, resulting Ca²⁺ influx from extracellular to intracellular. Ca²⁺ binds calcium calmodulin (CaM) and stimulates activation of calcium-stimulated signaling pathways. This process involves two pathways, adenylate cyclase and protein kinase, including calcium-calmodulin dependent protein kinase II (CaMKII) and

mitogen-activated protein kinase (MAPK). All of the process increase activation of NMDA receptor by higher expression of NR2B subunit.^{5,14,15}

After intrathecal administration of gabapentin and baclofen, there was an improvement on hyperalgesia condition by increasing latency time towards thermal stimulus. On day 12th, the time latency towards thermal stimulus was not significantly different from sham group. In addition to an impact of time latency toward thermal stimulus, gabapentin, and baclofen treatment provided positive influence on morphology of dorsal horn of spinal cord. Both of them reduced inflammatory cell (microglia) infiltration, but increased neuron body without prominent nucleolus and others glia cell and decreased vascular permeability compare to neuropathy group.

As previously explained, neuropathy caused by imbalance between inhibitory neurotransmitter (GABA) and excitatory neurotransmitter (Glutamate) leads to neuronal damaging. Gabapentin and Baclofen have been known as GABA agonist, drugs

that increase activity of GABA receptor with two different mechanism. Higher activation of GABA leads to increase the release of GABA and causes hiperpolarization, resulting decreases activity of NR2B subunit and lowering pain sensitization.¹⁶ According to Guo, et al¹⁷, the increased activity of NR2B subunit was associated with tyrosine phosphorylation that was occurred in chronic pain conditions. Furthermore, a dose response relationship has been demonstrated for these effects. In this research, dose gabapentin 100 nmol and baclofen 30 nmol give the biggest improvement in neuropathic pain.

Evidence suggests that patients receive treatment for diabetic neuropathy and post herpetic neuralgia with gabapentin, around 30% of patients can expect to achieve more than 50% pain relief. Around 30% of patients with neuropathic pain treated with gabapentin experienced minor adverse effect. However, in patients who in the same time suffer from both cancer and neuropathic pain, gabapentin showed higher response.¹⁸

Based on the findings of this research, gabapentin and baclofen can be considered to be an alternative treatment in neuropathy pain following the other diseases such as cancer, diabetes mellitus, infection and etc. This findings of this research also give an explanation why both of them can be used as first choice in neuropathic pain based on their mechanism in clinical condition.

Conclusion

Gabapentin and baclofen were effective treatments for neuropathic pain by recovered the histology in dorsal horn of spinal cord in neuropathic pain.

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