METHYLCOBALAMIN EFFECT ON MECHANICAL BEHAVIOR ALLODYNIA IN RATS *SPRAGUE DAWLEY*

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ABSTRACT

Neuropathic pain is an expression of the nerve damage that impaired its excitability, such as increased excitability in the nerve lesion and surrounding healthy nerves as well. It is estimated that the incidence of neuropathic pain ranges between 2-40% of all adult men. Some neuropathic pain can not be cured, such as in patients with diabetes mellitus, trigeminal neuralgia, cervical syndrome, carpal tunnel syndrome, lumbar stenosis, herniated nucleus pulposus and the carcinoma. The pathophysiology of neuropathic pain are complex and not fully understood. Methylcobalamin is a metabolite of vitamin B12 acts as a coenzyme in the formation of methionine from homocysteine. This reaction is useful in the formation of DNA, as well as maintenance of nerve function. Through the methylation reaction, methylcobalamin also plays a role in the formation of lecithin, a protein that plays an important role in the regeneration of peripheral nerves, including the formation of myelin. This study aimed to determine animal mechanical alodinia with or without the administration of methylcobalamin. Twenty male Sprague Dawley rats, aged 2 months, with an average weight of 150-250 g, obtained from the Laboratory LPPT Gadjah Mada University, Yogyakarta. The mice were randomly divided into 4 groups, each consisting of 5 mice. All the rats, their fifth lumbar nerve were ligated. Group I was the control mice (C) 0.9% NaCl given, group II (M1) were given a dose of 50 microg methylcobalamin, group III (M2) were given a dose of 100 microg methylcobalamin, while group IV (M3) were given a dose of 150 microg methylcobalamin 13 weeks provision. For 13 weeks, neuropathic pain behavior was assessed. Neuropathic pain behavior in experimental animals observed about onset, duration and filaments Von Frey numbers that cause mechanical alodinia. Result of the study showed methylcobalamin influence the mechanical alodinia. Based on the results obtained, it is concluded that there was a reduction in neuropathic pain in the methylcobalamin group compared with the control group.

Keywords: Lumbar V spinal nerve ligation, neuropathic pain, methylcobalamin.

Introduction

Neuropathic pain is a degenerative disease that affects many elderly group. An increase in the elderly population (aging population) will increase the prevalence of neuropathic pain in the group. These conditions will have an impact on health care costs, disruption of daily activities, emotional health, and decreased productivity of patients (Harden, 2005; Raja, 2005).

Degenerative disease of the nerve cells is the presence of a degeneration of nerve cells function without a known cause, from the normal state to a worse situation. The cause of the disease is often not known, including the group of diseases that are influenced by genetic factors, or at least occurs in one of the family members (familial factors), so often referred as *heredodegeneratif* disease. Cowers, in 1902, proposed the term *abiotrophy* for the disease, which means showed a decrease in resistance of neuronal cells and lead to premature death of nerve cells.

The International Association for the Study of Pain (IASP) defines neuropathic pain as pain that preceded or caused by a primary lesion or dysfunction of the nervous system, due to the central and peripheral nerve disorders. These disorders can be caused by compression, cuts,

infiltration, ischemic, ormetabolicdisturbancesofnerve cells, orany combination thereof. (Suharjani, 2010; Meliala, 2008).

The incidence ofneuropathic painis estimated to rangebetween2-40% ofalladultmen. In the United Statesthe number ofcases ofpatients withneuropathic painis estimated toreach3.75milliontotalcasesofchronicneuropathic pain, including thatcausedbycarcinoma, spinal cordinjury, back painandphantompain. Recordsof healthinsuranceinthe USA show recurrentpainfromlow back painandfacial painabout 45% of theentirerecord ofthe existingpain. In the UK, approximately25% ofpatientsinpainclinicssuffer fromneuropathic pain. (Harden, 2006).

Other reports suggestedthatneuropathic painfoundone sixthofthe total population(Xie, 2005). The majority ofpatients withneuropathic painexperience painthroughout his life, this is becausethe causes ofthepaincannot becured, such asdiabetesmellitus, trigeminal neuralgia, cervicalsyndrome, carpaltunnelsyndrome, lumbarstenosis, herniatednucleus pulposusandcarcinoma.

Pathophysiology ofneuropathic painis verycomplexandnot yetfully known. Many theories have beenproposed, butnot fully understood. The existence ofinjury toperipheralnerveconductionof painwilllead toabnormalactivityofafferentfibers, namelyectopic discharge. The presence ofectopic dischargecan causecontinuouspain, resulting inperipheralandcentral sensitization. (Baron, 2009; Decosterd & Berta, 2009; Ossipov & Porreca, 2009; Dupere *et al.*, 2005; McMahon *et al.*, 2004; Dworkin *et al.*, 2003; Meliala & Barus, 2003; Bridgs *et al.*, 2001).

Carbamazepineis a drugthat iswidely usedfor the treatment ofneuropathic pain. Other drugsthat are widely usedfor the treatment ofneuropathic painareamitriptyline, gabapentin, pregabalin, tramadol, and others. Drugsareonlyforsymptomatictreatmentand theyhaveside effects such asheadache, dizziness, vertigo, dry mouth, nausea, vomiting, and evencan causeallergic reactionssuch asStevenJohnsonSyndrome (Baron, 2009; Decosterd dan Berta, 2009; Ossipov & Porreca, 2009; Dupere *et al.*, 2005; McMahon *et al.*, 2004; Dworkin *et al.*, 2003; Meliala, 2008; Bridgs *et al.*, 2001). The use ofthedrugsfor the treatment ofneuropathic painis stillnot satisfactory, onlylessthan50% of patientswhoimproved(Bridgs etal., 2001;Suharjani, 2010), whichmeans thatso far no drugwascapable ofgivesatisfactory resultsagainstneuropathic pain. On theother handmanystudiesfoundevidence which indicatesregressionof painin patients withneuropathic pain who weregivenmethylcobalamin, such astrigeminal neuralgia, occipitalneuralgia, post-herpetic neuralgia. Administration ofmethylcobalaminalsoreduces painin cases oflow back paincausedbylumbar-spinal canal stenosis, lumbarspondylosis, sciaticaradicularneuralgia, andherniatednucleus pulposus (Meliala & Barus, 2008).

Methylcobalamin is a metabolite of vitamin B12 that acts as a coenzyme in the formation of methionine from homocysteine. This reaction is useful in the formation of DNA, as well as maintenance of nerve cell function. Through the methylation reaction, methylcobalamin also plays a role in the formation of lecithin, a protein that plays an important role in the regeneration of peripheral nerves including myelin formation (Björkegren, 2008; Meliala and Barus, 2008; Kräutler,1998).

Based on the facts above, the researcher is interested in knowing the effect of methylcobalamin on neuropathic pain behavior in Sprague Dawley rats were ligated fifth lumbar spinal nerves as an alternative in the primary treatment of neuropathic pain.

Materials and Methods

This studyis an experimental studyin animalswithmethylcobalaminas theindependent variableandneuropathicpainbehavioras thedependentvariable. TwentySpragueDawleyrats, aged2months, witha meanweight of150-250grams, which are obtained fromLPPTLaboratory

ofGadjah Mada University, Yogyakarta, usedin this study. Rats were adapted totheexperimentalcagefor 1 weekwithstandardfeedconsumptionandwaterad libitum. After theadaptation periodis complete, the 5thlumbarnerveof mice were ligatedusingthe same methodand thenwere randomly dividedinto 4groupsconsist of5 rats in each group.

Group Iwasthe controlrats(C) weregiven0.9% NaClas scheduledadministration ofmethylcobalaminIMintramuscular(IM) in theothergroups of mice. Group II(M1) is themice treatedwith the50mgdosemethylcobalaminIM2x/week, groupIII(M2) is themice treatedwitha dose of 100mcgmethylcobalamin, whilegroupIV(M3) israts givena dose of 150mcgmethylcobalamininintramuscular2x/week.

In summary, the study designcan be seenin Table1below.Examination ofneuropathic painbehaviorsof Sprague Dawleyis the examination ofmechanicalallodynia. Assessment include; onset arise, length,andvonFreyfilamentnumbersthatcausemechanicalallodynia.

Table I : Table Design Study

Groups	Treatment				
	Surgery	SNL (spinal nerve ligation)	methylcobalamin (IM 2 times a week)	NaCl 0.9% (IM 2 times a week)	Parameter analysis
K	+	+	-	+	+
M1	+	+	50 µg	-	+
M2	+	+	100 µg	-	+
M3	+	+	150 µg	-	+

Rats were maintained for 13 weeks, observations were made of the behavior associated with pain using von Frey filaments on the first, third and fifth day, after the fifth lumbar nerve ligation, and at the end of each week.

Mechanical hypersensitivity was assessed using von Frey filaments gradation series. Von Frey filaments were slowly pressed perpendicularly into the skin of experimental animals, until the filament began to bend. The first assessment was done on the leg that had the procedure SNL and then on a healthy foot. The frequency of the stimulus was done about 1 times per second and each intensity performed 5-10 times. The assessment was conducted on the day 1, 3, 5, 7, 14, and then each end of next week according to research that was done by Persson (2009). Comparisons between groups of neuropathic pain behavior in experimental animals were analyzed using ANOVA.

Results and Discussion

Onset of mechanical allodynia

The results of the initial examination of the onset of mechanical allodynia in each group is different. Mechanical allodynia in Group K appears on average at day 5.4 (SD 1.67, minimum 3, maximum 7), the average M1 group at day 4.6 (SD 2.19; minimum 3, maximum 7), the average M2 group on day 9 (SD 4.64; minimum of 5, maximum of 14) and M3 on average 8.6 days (SD 4.6; minimum of 5, maximum of 14).

In group M2 and M3 was found the average initial onset of mechanical allodynia longer than the group K and M1, whereas in the M1 group was found the average start earlier onset of mechanical allodynia of the group K, it can be concluded that, descriptively, methylcobalamin can prolong the onset of mechanical allodynia, the dose of methylcobalamin that was given in group M2 is to the dose that has been quite effective in prolonging the onset of mechanical allodynia.

Statistical analysis that were conducted on group K and M2 showed no significant difference (p = 0.141), this was because the number of samples from each group is few and data of the first onset of mechanical allodynia highly varies (wide range) either in each group and between groups. The reason of statistical analysis was conducted among a group of K with group M2 due to in the M2 group there were some differences in the initial onset of mechanical allodynia.

This study showed differences on the first onset of mechanical allodynia in rats from each group. The average of the first onset of mechanical allodynia (descriptively) group K earlier than M2 and M3 groups, but the average group M1 is lower than the average group of K. This suggests that the dose of methylcobalamin was given to the M1 does not slow the onset of mechanical allodynia, methylcobalamin dosage given in the M2 group was able to slow the onset of mechanical allodynia.

The given dose of methylcobalamin in M3 group was able to slow the onset of mechanical allodynia compared to group K and M1 but not able to slow the onset of mechanical allodynia compared to the average in the M2 group. This fact showed that a given dose methylcobalamin on M2 group has been effective in slowing the onset of mechanical allodynia.

On the third day, the rats in group M1 raised more mechanical allodynia than in the control group. This were due to 1) the dose in M1 group is not effective to slow the onset of mechanical allodynia, 2) length of exposure to methylcobalamin on rat in M1 group is very short (only 3 days), 3) nerve regeneration phase on day three finishes, cleaning debris by microglia phase and has not yet occurred sinaptogenesis (Colman & Carl, 1999) so that the administration of methylcobalamin has not been influential in the regeneration of peripheral nerve cells.

On the seventh day, rats in groups M2 and M3 experiencing mechanical allodynia by the same amount (3 mice). This condition indicates that the dose methylcobalamin on M3 group was not able to slow the onset of mechanical allodynia compared to M2 group. It is also seen in the mean onset of mechanical allodynia M3 group was not higher than the mean onset of mechanical allodynia group M2, furthermore M3 group mean even lower than the mean of the group M2. It is explained that the increase in dose of methylcobalamin on M3 group was not able to increase the difference in slowing the onset of mechanical allodynia of the group M2. The same phenomenon is also seen between the groups K with M1 group, showed an increase in dose of 50 mg of methylcobalamin was not able to slow the onset of mechanical allodynia, but the difference in dose of 100 mg was able to slow the onset of mechanical allodynia.

Mechanical allodynia results from hyperexcitability of the nervous system. Hyperexcitability caused by ectopic discharge that occurs because of the accumulation of the neurotransmitter VGSC activity along the newly formed lesion area that ultimately arise ectopic generator. Continuous ectopic generator sends impulses to the dorsal ganglion radix, to the dorsal horn and finally to the central nervous system that will cause peripheral and central sensitization. Finally, the process arises mechanical allodynia (Baron, 2009; Cordorre, 2009).

Onset of mechanical allodynia varies, as does sprouting and neuroma formation, ie within a few hours to several days (Meliala, 2008; Dunteman, 2004; Attlal, 2000; Devor &Seltzer, 1999). Due to the time span of sprouting and neuroma formation is very wide (several hours to several days), this led to the emergence of mechanical allodynia was also highly varies, so that the data appears very varies and it is not able to show statistically significant results.

The duration of Mechanical Allodynia

The results of examination of the duration of mechanical allodynia within group K are: first rat 6 weeks 3 days, second rat 5 weeks 3 days, third rat 5 weeks 5 days, fourth rat 6

weeks, and fifth rat 7 weeks 5 days. In the M1 group, the duration of mechanical allodynia in first rats 6 weeks 5 days, second rat 1 week 5 days, third rat 8 weeks 3 days, fourth rat 5 weeks 5 days, fifth rat 4 weeks 5 days.

In group M2 was observed duration of mechanical allodynia in first rat first 2 weeks, second rat 5-week, third rat 5 weeks 3 days, fourth rat 2 weeks, fifth rat 7 weeks 3 days. Moreover,in the group of M3 was obtained duration of mechanical allodynia in first rat 5 weeks 3 days, second rat 5 weeks 5 days, third rat 3 weeks,fourth rat 3 weeks, fourth rat five 4 weeks.

The whole rats (100%) in group K experienced mechanical allodynia until day 35, all mice (100%) in the M1 group experienced mechanical allodynia until day 12, all mice (100%) in the M2 group experienced mechanical allodynia until day 14 and all mice (100%) in the M3 group experienced mechanical allodynia until day 21.

On day 14, 21, 28 and 35 post-SNL seemed decline in the number of mice that experienced mechanical allodynia in groups M1 and M2 than M3 group K. In the group of M3 all the rats were still experiencing mechanical allodynia at day 14 post-SNL, whereas on day 21 and day 35 the number of mice with mechanical allodynia was similar to the M2. At day 28 post-SNL it was observed a decrease in the number of mice that continued to decline ranging from group K. On day 42 post-SNL there was no difference in the number of mice that still experienced mechanical allodynia between group K with group M1. On day 49 there was no difference in the number of mice that still experienced mechanical allodynia in group K, M1 and M2.

On day 60, in all groups, none of mice (K, M1, M2, and M3) experienced the mechanical allodynia (Figure 1). If followed from the number of mice with mechanical allodynia of each group, it is shown that all of mice in the control group still experienced mechanical allodynia until day 35 post-SNL, while in the groups M1, M2, and M3 there were rats that did not experience mechanical allodynia. The average duration of mechanical allodynia experience among rats in group K was 5 weeks 1 day 9 hours 36 minutes. In addition, the average duration of mechanical allodynia experience among rats in group M1 was 5 weeks 3 1/5 day. Furthermore, rats in group M2 that experienced mechanical allodynia was 4 weeks 2 days 14 hours. Lastly, rats in group M3 experienced mechanical allodynia was 3 weeks 5 days.

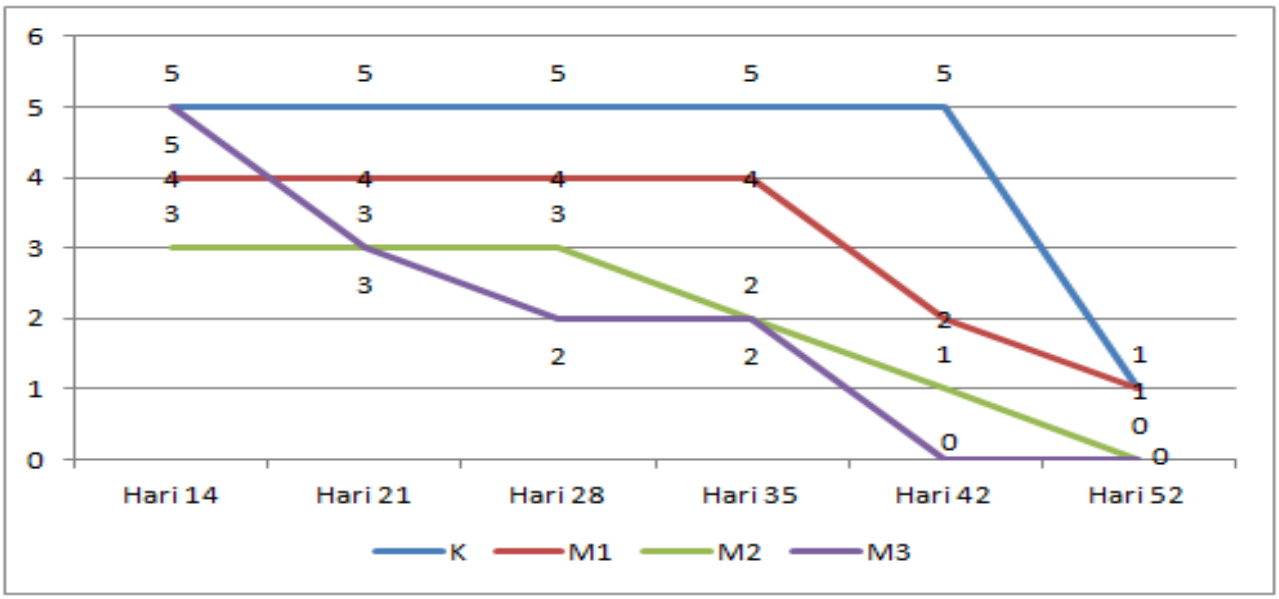

Figure 1 Graph the number of mice in each group that experienced mechanical allodynia by day post-SNL. C = control, methylcobalamin M1 = 1, M2 = methylcobalamin 2, M3 = methylcobalamin 3.

The average duration of mechanical allodynia in groups M2 and M3 is lower than the group K. The effective dose to shorten the duration of mechanical allodynia was the dose of methylcobalamin in groups M2 and M3. Statistical analysis in all four groups showed no significant difference ($p < 0.05$), statistical analysis between the two groups (K with M1, K with M2, K with M3) showed significant differences between the groups K with M3 ($p = 0.027$).

In this study, the duration of mechanical allodynia in rats from each group was different each time. The average time of mechanical allodynia (descriptively) in group K longer than the group M1, M2 and M3. This indicates that methylcobalamin can shorten the time of mechanical allodynia experienced by rats in group M1, M2 and M3, in addition, the dose of methylcobalamin also influences in shortening the duration of mechanical allodynia. Statistically significant only showed between group K with the M3 difference ($p = 0.027$).

The duration of mechanical allodynia depends on the amount of sprouting and neuroma formed at the area of injury, the process of regeneration and factors arising after inhibition after nerve injury. In this study, the nerve injury, that was done, is the same in all rats of all groups SNL left lumbar 5. The difference is whether there is an effect on the administration of methylcobalamin to nerve regeneration process as already described above. Methylcobalamin role in the synthesis of proteins for the regeneration of damaged nerve cells will shorten the duration of mechanical allodynia.

Von Frey filament numbers that cause mechanical allodynia

Von Frey filament numbers that cause mechanical allodynia in the control group was lower than the group M1, M2 and M3. It is also proved by statistically significant difference ($p = 0.007$) among the four groups. Descriptively and statistically showed a significant difference ($p = 0.007$) von Frey filament numbers that cause mechanical allodynia..

These conditions indicate that 1) methylcobalamin influences mechanical strength that is given to induce mechanical allodynia, 2) the higher the dose of methylcobalamin the greater the mechanical strength required to cause mechanical allodynia.

Nerve ligation would cause mechanosensitive-hot-spot, which is very sensitive to mechanical stimuli, so that with a little knock will cause pain at sensory areas (Baron, 2009; Decosterd & Berta, 2009; Ossipov and Porreca, 2009; Meliala, 2008; McMahon et al., 2004; Dupere et al., 2003; Bridges, 2001). Biochemical processes that occur as a result of administration of methylcobalamin, the same as in the previous discussions, in which protein synthesis occurs very useful in the regeneration of neural structures, particularly peripheral nerve.

Conclusion

Methylcobalamin can slow the onset of mechanical allodynia, and methylcobalamin dosage given was also influential in slowing the onset of mechanical allodynia, mechanical allodynia duration, and number of filaments. This study found a decrease in mechanical allodynia experimental animals after nerve injury in the methylcobalamin group compared with the control group.

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