Human & Veterinary Medicine
International Journal of the Bioflux Society HVM

OPEN ACCESS
Short communication

A preliminary study on the effect of methylcobalamin application on reducing neuropathic pain

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Abstract: Aim: The aim of this present study was evaluate the effect of methylcobalamin on the sprouting expression post nervus spinalis lumbal V ligation. The sprouting expression is an important parameter in neuropathic pain treatment. Materials and method: A total of 20 Sprague Dawley mice were used in this study. The mice were divided into four groups; the first group was the control group (without methylcobalamin administration) and the other three groups were administered with methylcobalamin at doses of 50µg, 100µg and 150µg, respectively. After 14 weeks, the mice were ligated and their nervus spinalis lumbal V were prepared for histological examination. Results and discussion: The study revealed that methylcobalamin treatment was succesful in decreasing sprouting, this indicates that there had been a process of regeneration of nerve cells in the mice administered with methylcobalamin. Conclusion: methylcobalamin administration has given a positive impact on the reducing neuropathic pain of Sprague Dawley mice.

Key Words: sprouting, nervus spinalis, methylcobalamin, neuropathic pain.

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Introduction

Sprouting is the development of new branches of axon and it is an indication of axon damaged. The new branches of this acson are derived from the parental axons which are still associated with the cells body. It is the connection between damaged fibers to healthy nerve fibers (Cotman 1999). However, some sprouting were not connected to the parental nerves, they would develop a neuroma with a new neurotransmitter receptors existing inside, and natrium, kalium and calcium ions canals (Zimmermann 2001) causing increases of excitability or hypersensitivity (Meliala 2008). This then generates the ectopic discharge of neuropathic pain, alongside inflammation as another factor stimulating the pain. Nowadays, no neuropathic pain treatments are successful in relieving the pain. Presently, the administration of anti-pain medication is a common treatment for neuropathic pain patients; in which this treatment can reduce the pain to less than 50% (Suharjanti 2010).

Based on the protocol of neuropathic pain treatments, tricyclic is a common medication for the pain. This drug is an anti-depressant and anti-epilepsy with some negative side effects such as nausea, vomiting and dizziness. These symptoms may reduce the productivity of the patients. Therefore, it is still a challenge to find the most effective medication for neuropathic pain, and one of the alternative drugs is methylcobalamin.

Methylcobalamin is coenzyme forms of cobalamin (vitamin B12), this coenzyme has an important role as cofactor in the enzyme methionine synthase process which functions to transfer the group of methyl to regenerate of methionine from homocysteine (Banerjee & Ragsdale 2003; Toohey 2006). The researchers believe that methylcobalamin has no negative side effects on treated patient. The previous studies showed that methylcobalamin has the ability to regenerate nerve cells (Inada et al 1981; Mizukami et al 2011). It has been applied to treat stenosis lumbalis, neuralgia sciatica, and servicalis syndrome (Meliala & Barus 2008); These studies showed that pain was reduced up to 50%-65% (Meliala & Barus 2008). However, thus far the expression of ligation nervus spinalis lumbal from the administration of methylcobalamin has not been investigated. We speculate that the regenerated nerve cells would give a positive effect to eliminate the sprouting process then further reduces pain. However, some studies reported that the administration of methylcobalamin would generate wallerian and fibroblast perineural (Lee & Wolfe, 2000), but the pathological features of wallerian degeneration and perineural fibroblasts were not associated with neuropathic pain (Byers & Bonica 2001), therefore, these issues were ignored in this study. Nonetheless, the objective of this present study was to describe the effect of methylcobalamin on the sprouting feature and the experimental

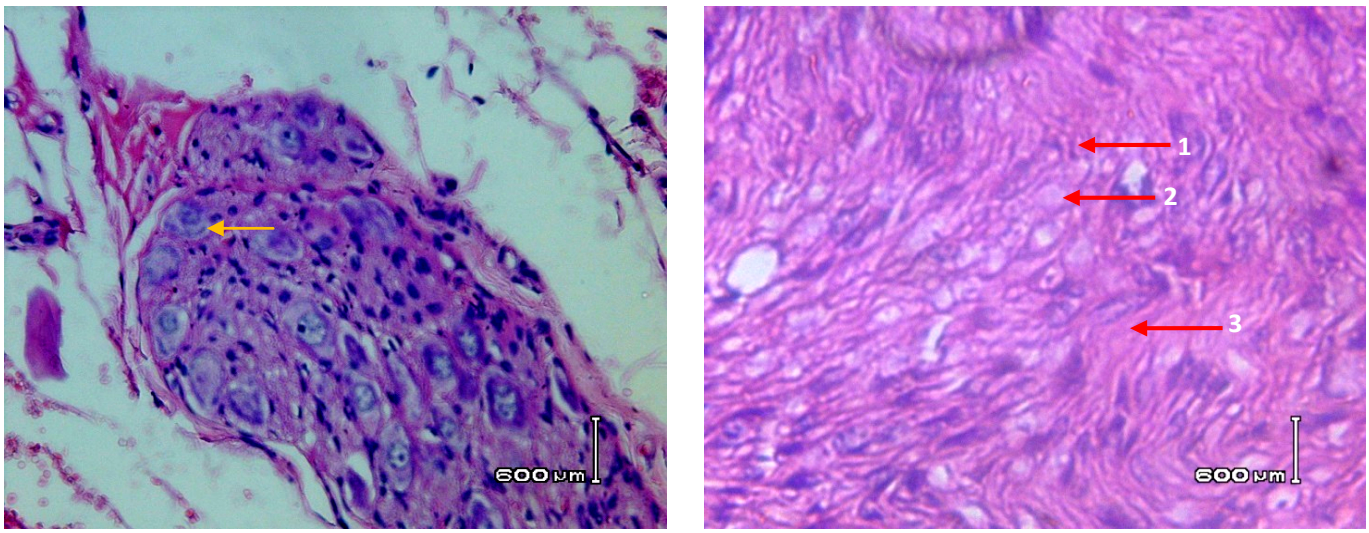

Figure 1. (a). Histological apperance of nervus spinalis lumbalis V at the pre-ligasi area of the control group, the arrow is hypertropy nerve cell (1000x magnification); (b) the histological appearance of the treated group of mice with (1) acsonal sprouting, (2) wallerian degeneration, and (3) perineural fibroblast.

Sprague Dawley mice were used as a model. It further aims to portray this effect as an important parameter in treating neuropathic pain.

Materials and Methods

Two month old Sprague Dawley mice with the body weight ranged between 150-250 g were used in this experiment. The mice were collected from Gadjah Mada University, Yogyakarta, Indonesia. A total of 20 mice were adapted in the experimental cage for 7 days and they were fed with a commercial diet, ad libitum. They were divided into four groups and every group comprised five mice which were selected randomly. The nervus spinalis lumbal IV of all mice were ligated.

The first group of mice (K) was administered with NaCl 0.9% as the control group (K); the second group of mice was administered with 50µg of methylcobalamin (M1); the third group of mice was administered with 100µg of methylcobalamin (M2); the fourth group was administered with 150µg of methylcobalamin (M3). The experimental drug was intramuscularly administered at a frequency of two times a week or three days interval (Teramoto et al 1984) for 14 weeks. After 14 weeks, the mice were anesthetized with MS.222 over doses and then dissected. Ligated nerve tissues were taken for histological examination. The tissue samples were soaked in xylene and embedded in paraffin. Afterwards, they were sectioned on a microtome. The 2-3 micron sections were moved on a slide, then kept in the oven for 24 hours. After 24 hours, the samples were stained with Ehrlich hematoxilline and eosine. Subsequently, they were observed under the compound light microscope at 1,000X magnification (Muchlisin et al 2010). The study has followed the standard of animal ethic for research purposes of Syiah Kuala University.

The spouting appearance was categorized into five levels: no expression (-), light expression (+), moderate expression (++), high expression (+++), and very high expression (++++). The data were statistically analyzed by the Kruskal Wallis test at a 5% significance level using the Statistical Analysis System software Version 9.1.3 (SAS Institute Inc., Cary, USA).

Results and Discussion

A pathologic appearance of neuron cells hypertrophy was found (see Figure 1a). The wallerian degeneration, spouting, and fibroblast perineural was found to also occur (see Figure 1b). In the control group, approximately 20% of the mice had (++),40% had (+), and another 40% had no sprouting. All mice samples in the second group (M1) showed no appearance of sprouting. However, about 20% of the mice had (++) and 80% had no sprouting in the third group (M2). In addition, all mice in the fourth group had no sprouting occurrence (Table 1).

The Kruskall-Wallis test revealed that there was no significant effect of methylcobalamin doses on the sprouting expression of ligasi nervus spinalis lumbal 5 on the experimental mice ($P>0.05$). Accordingly, the mice administered with methylcobalamin gave better results compared to the control group (without methylcobalamin). This indicates that methylcobalamin application gave a positive respond in reducing neuropathic pain.

Table 1. Total of sprouting expression in the control and treated group of mice. Note: no expression (-), light expression (+), moderate expression (++), high expression (+++), very high expression (++++)

Group	++++	+++	++	+	-
Control	0	0	1	2	2
50 µg of methylcobalamin	0	0	0	0	5
100 µg of methylcobalamin	0	0	1	0	4
150 µg of methylcobalamin	0	0	0	0	5

The study further revealed that methylcobalamin treatment was succesful in decreasing the sprouting. This indicates that there has been a process of regeneration of nerve cells in the mice administered with methylcobalamin, and thus believed to have an impact on pain reduction (McMahon & Cafferty 2004). The

results of this study is in agreement with the previous reports. For example Mizukami et al (2011) reported that methylcobalamin could stimulate DNA recovery of nerve cells through protein and lysitine synthesis, which are the primary compound of myelin. Moreover, Inada et al (1981) stated that in the damaged nerve tissue, methylcobalamin would accelerate the synthesis of nucleic acids and proteins in nerve cells. In addition, methylcobalamin also will encourage the process of myelination and lipid transport where protein and lipid are the important materials for axon regeneration.

Hence, methylcobalamin plays an important role as a coenzyme for methionine synthesis derivated from homocysteine. In a negative condition of methionine balance, homocysteine is converted into methionine by receiving a methyl group from 5 methyltetrahydrofolate through the action of methionine synthase (Zhang & Ning 2008). This process is crucial for thymine development of a nucleic acid which is useful in protein synthesis. Furthermore, the study suggests that methylcobalamin treatment gave a positive impact in reducing neuropathic pain as showed in the histological feature of spinal lumbalis V compared to without methylcobalamin. However, there was no quantitative difference among the groups. This was possibly due to sprouting which only occurred at nerve cells C, and no sprouting in nerve cells were detected. Nevertheless, the crucial evidence confirmed in this study is that the treated group had sprouting appearance resulted in Voltage Gate Sodium Channel (VGSC) accumulation as an important indicator for cell degeneration (Zimmermann 2001).

Conclusion

This study concludes that the sprouting expression of treated mice with methylcobalamin was lower compared to the control group (without methylcobalamin). However, there was no difference in sprouting expression between the treated groups.

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Citation	Mutiawati E, Meliala L, Aji D, Wasito W, Muchlisin ZA. A preliminary study on the effect of methylcobalamin application on reducing neuropathic pain. HVM Bioflux 2015;7(2):101-103.
Editor	Stefan C. Vesa
Received	14 March 2015
Accepted	29 April 2015
Published Online	5 May 2015
Funding	None reported
Conflicts/ Competing Interests	None reported