Early Co-Administration of Vitamin E Acetate and Methylcobalamin Improves Thermal Hyperalgesia Following Partial Sciatic Nerve Ligation In Rats.

Aashish S. Morani*, Aniket Nikam and Subhash L. Bodhankar

Department of Pharmacology, Poona College of Pharmacy, Paud road, Kothrud, Pune 38,India

Summary

To study the effect of early co-administeration of vitamin E acetate and methylcobalamin on thermal hyperalgesia following partial sciatic nerve ligation in rats.

Mononeuropathy was induced by Partial sciatic nerve ligation (PSNL) in Wistar rats. Treatment with vitamin E acetate (50 mg/kg, intraperitoneal i.p.; VE) and methylcobalamin (500µg/kg, i.p.; MCA) begun from day 2 post surgery. The effect of co-administered VE and MCA (MVE) was observed on the paw withdrawal latency (PWL) behaviour to heat stimuli on day 0, 2, 5, 10 and 15.

A significant reduction in PWL was observed on day 2 for untreated (UN) and MVE group of animals ($p<0.01$). This was followed by a gradual improvement in PWL in MVE treated animals indicating the decrease in pain threshold following early exposure to MVE which was not a case in UN group of animals. Early treatment with MVE improved PWL to thermal stimuli in rats with partial sciatic nerve ligation injury.

Key-words: Monuneuropathy, partial sciatic nerve ligation, vitamin E acetate, methylcobalamin, thermal hyperalgesia

Running tittle: Early co-administration of vitamin E and methylcobalamin in sciatic nerve ligation injury.

Correspondance to: Aashish S. Morani
aashishmorani@gmail.com

Introduction

Partial ligation of peripheral nerves in laboratory animals mimics symptoms produced in causalgic humans. Additionally, the nerve trapped in the ligature produces the spontaneous pain which can be observed as an exaggerated response to non-noxious stimuli (hyperalgesia) or low threshold stimuli (allodynia). [1] These behavioural sensations can be well attributed to the inflammatory reactions triggered by the nerve insult. Collectively, it results into the production and accumulation of the reactive oxidant species (ROS) which further assists in the progression of neuropathic pain. [2,3] Our previous findings demonstrate that early treatment with VE [4] and MCA [5] attenuated neuropathic pain in rats. Here, we aimed at studying the effect of early co-administration of MVE on thermal hyperalgesia (TH) produced by PSNL in rats.

Materials and Methods

Animals and treatment

24 male Wistar rats (200-225 g) procured from National Toxicology Centre, Pune, were used for the present study. These animals were housed under standard animal house conditions (24±2° C, 70% relative humidity, 12hr light: dark cycle) with free access to food and water except during surgery and testing. Animals were divided randomly into four groups. The MVE group were treated with VE (50 mg/kg, i.p.) followed by MCA (500 µg/kg, i.p.) from day 2 post surgery. Control, sham and UN group of animals were treated with vehicle.

TH was assessed on day 0 (just before surgery), day 2 (before MVE/vehicle treatment), day 5, day 10 and day 15. All experiments were carried out between 0900 – 1700 hrs followed by the MVE/vehicle treatment. The care and maintenance of animals was as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals in India. All experimental procedures were reviewed and approved by Institutional Animal Ethics Committee.

Surgery

Under deep anaesthesia produced by pentobarbital sodium (50 mg/kg, i.p.), the right sciatic nerve of 12 animals were ligated. Using an iris forceps, the right sciatic nerve was carefully exposed at the right gluteal region and a 4.0 silk suture was inserted into the nerve and tightly ligated so that the dorsal 1/3 – ½ of the nerve thickness was trapped in the ligature. The incision site was closed. All the surgical procedures were performed under sterile operative conditions. 6 animals from this group were treated with vehicle (UN) whereas other 6 rats were treated with MVE. In sham (n=6) group of animals, the right sciatic nerve was exposed but was left intact. Both control (n=6) and sham group of animals were treated with vehicle. Treatments for all groups of animals begin from day 2 onwards.

Thermal Hyperalgesia (TH)

TH was assessed using Ugo Basile Hot Plate Analgesiometer (Versace, Italy). The hot plate was maintained at 55±0.1° C and the paw withdrawal latency (PWL) in seconds (s) to the thermal stimulus was determined. A cut off time of 22 s was set to avoid tissue damage. The test was repeated thrice at an interval of 15 min and each time was carried out by a different blinded observer. A mean of these readings was taken as final response.

Drugs

Methylcobalamin and pentobarbital sodium (gifted by Emcure Pharmaceuticals, Pune, India) were dissolved in physiological saline. Vitamin E acetate (Hi Media, Mumbai, India) was suspended in 1% Tween 80 (Loba Cheme, Mumbai, India). i.p. injections were in the volume of 1 ml/kg. All drug weights refer to the salt.

Statistical analysis

PWL were expressed as mean ± SEM. Statistical significance between groups was determined by one-way ANOVA's followed by Tukey post-hoc comparisons. Data were considered significant at $p<0.05$. All statistical analysis were performed using Graphpad-Prism (San Diego, CA, USA)

Results and Discussion

On day 2 post surgery, significant reduction in PWL to thermal stimulus was observed in UN and MVE group of animals which however was not the case for sham and control group [$F(3,20)=11.96$, $p<0.001$]. Thus confirming the presence of neuropathic pain due to PSNL. Although a significant decrease in latency was noted on 5^{th} day in MVE group of animals [$F(3,20)=13.16$, $p<0.0001$], a gradual improvement in PWL was observed on 10^{th} [$F(3,20)=4.77$, $p=0.012$] and 15^{th} [$F(3,20)=4.08$, $p=0.021$] day post surgery, which was comparable to sham. However, this difference persisted throughout for UN group indicating the presence of TH. (Table.1)

Our previous observations show that early treatment with both VE [5] and MCA [6] for 27 days led to a recovery from TH comparable to sham group due to PSNL in rats. The current study clearly indicate that under similar experimental conditions, co-administration of MVE for 14 days resulted into a quick onset of recovery (day 5) and an early improvement in TH comparable to sham (day 10) as compared to animals treated with either VE or MCA alone. This behavioural effect of co-administered MVE might be due to the anti-oxidant property of VE [5, 7] and neuro-protective effect of MCA. [6] However, further investigations are required to understand the mechanisms by which these agents act.

Table.1 Effect of co-administered vitamin E acetate (50 mg/kg, i.p.) and methylcobalamin (500 µg/kg, i.p.) on paw withdrawal latency (in seconds) in partial sciatic nerve ligation in rats.

	Day 0	***Day 2***	***Day 5***	***Day 10***	***Day 15***
Control	14.23±2.1	15.32±3.0	15.9±2.6	16.8±4.1	17.5±3.6
Sham	15.6±1.6	17.5±2.6	18.9±1.3	16.7±2.1	17.5±3.2
UN	16.5±2.3	4.7±0.3***	6.2±0.84***	5.8±0.24*	6.81±0.9*
MVE	15.4±2.5	4.2±0.61***	10.8±0.63**	14.4±1.2	15.6±1.24

UN= PSNL+ vehicle treated, MVE= PSNL+ VE and MCA treated. Paw withdrawal latency expressed as mean ± SEM. Statistical analysis were performed by applying one way ANOVA's followed by Tukey post-hoc comparisons. *p<0.05, **p<0.01, p<0.001. Data compared to sham. N=6 for each group.

Acknowledgements

The authors are thankful to Emcure Pharmaceuticals, Pune for providing gift samples of methylcobalamin and pentobarbital sodium. The authors are also thankful to Mr Amol Muthal for his technical assistance.

References

1. Bridges D, Thompson SW, Rice AS: Mechanisms of neuropathic pain. Br J Anaesth, 2001, 87, 12-26.
2. Bagdatoglu C, Saray A, Surucu HS, Ozturk H, Tamer L: Effect of trapidil in ischemia/reperfusion injury of peripheral nerves. Neurosurgery, 2002, 51, 212-219, discussion 219-220.
3. Kurtoglu Z, Ozturk AH, Bagdatoglu C, Turac A, Camdeviren H, Uzmansel D, et al: Effect of trapidil on the sciatic nerve with crush injury: a light microscopic study. Neuroanatomy, 2004, 3, 54-58.
4. Kurtoglu Z, Ozturk AH, Bagdatoglu C, Polat G, Aktekin M, Uzmansel D, Camdeviren H, Bagdatoglu O, Sargon M: Effects of trapidil after crush injury in peripheral nerve. Acta Med Okayama, 2005, 59, 37-44.
5. Morani AS, Bodhankar SL: Neuroprotective effect of vitamin E acetate in models of mononeuropathy in rats. Neuroanatomy, 2008, 7, 33-37.
6. Muthal AP, Morani AS, Bodhankar SL: Neuroprotective Effect of ultra-high dose of methylcobalamin in models of mononeuropathy in rats. Pharmacologyonline, 2008, 2, 282-295.
7. Al Moutaery K, Arshaduddin M, Tariq M, Al Deeb S: Functional recovery and vitamin E level following sciatic nerve crush injury in normal and diabetic rats. Int J Neurosci, 1998, 96, 245-254.