Pharmacological Reports
2010, 62, 405–409
ISSN 1734-1140

Short communication

Early co-administration of vitamin E acetate and methylcobalamin improves thermal hyperalgesia and motor nerve conduction velocity following sciatic nerve crush injury in rats

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Abstract:
Our previous studies have shown that early administration of vitamin E acetate (50 mg/kg, *ip* (VEA)) and methylcobalamin (500 µg/kg, *ip* (MCA)) for 30 days improved conduction velocity and neuropathic pain behavior. Here, we evaluated the effect of early co-administration of VEA and MCA (MVE) on thermal hyperlagesia (TH) and motor nerve conduction velocity (MNCV) in rats with sciatic nerve crush injury (SNCI). Fifteen days post-surgery, a reduction in paw withdrawal latency (PWL) was observed in untreated (UNTR) rats. However, latency improved in MVE-treated animals, comparable to the placebo. On day 15, a decrease in MNCV was observed in the UNTR group of animals, and this effect was not observed for the MVE and placebo groups of animals. The results of this study indicate that early exposure to MVE attenuates the progression of TH and improves MNCV in rats with SNCI.

Key words:
vitamin E acetate, methylcobalamin, sciatic nerve crush injury, neuropathic pain, thermal hyperalgesia, motor nerve conduction velocity

Abbreviations: *ip* – intraperitoneal, I/R – ischemia/reperfusion, MCA – methylcobalamin, MNCV – motor nerve conduction velocity, MVE – co-administered VEA and MCA, PWL – paw withdrawal latency, SNCI – sciatic nerve crush injury, TH – thermal hyperalgesia, UNTR – untreated group of animals, VE – vitamin E, VEA – vitamin E acetate,

Introduction

Mechanical nerve crush in animals produces hyperalgesia (increased response to noxious stimuli) and allodynia (pain response to a low threshold stimulus), which resembles the symptoms produced in causalgic humans [8, 26, 33]. Nerve compression followed by ischemia results in demyelination, axonal degeneration, and impaired nerve blood flow and reflow, all of which lead to endothelial edema, agranulocyte plugs and microvascular thrombosis. Collectively, these events cause continuous fiber injury. Furthermore, the release of toxins from neutrophils and macrophages impairs tissue protection by accumulating reactive oxidant species (ROS). These accumulated ROS aggravate tissue destruction and assist in the development and maintenance of neuropathic pain [5, 12, 13,

15, 16, 26, 34]. Along these same lines, free radical scavengers have been shown to attenuate the development of neuropathic pain [3, 18]. Thus, the administration of pharmacological agents that reduce the concentration of these neurotropic factors after ischemia/reperfusion (I/R) injury may protect the peripheral nerves from further damage [1, 5, 13, 24].

Vitamin E (VE) is one of the most effective antioxidants found in the human biological system [3]. Recent studies have shown that treatment with VE alone [23], or in combination with other antioxidants [1, 2, 9], decreased oxidant stress after I/R injury in various tissues. Additionally, VE has also been shown to improve nerve electrophysiology [18, 24]. Clinically, VE supplements prevented the progression of cisplatin neurotoxicity [20].

MCA has been reported to prevent nerve degeneration in diabetic-, acrylamide- and surgery-induced neuropathies in laboratory animals [17, 19, 28, 30]. Clinically, MCA prevented neuropathic complications in uremic patients [14], improved compound muscle action potentials in patients with amyotrophic lateral sclerosis [10, 11], and prevented pain due to diabetic neuropathy [25].

Combination of antioxidants with VE has shown to improve nerve function in experimental neuropathies [24]. The aim of our current study was to evaluate the effect of early co-administration of MVE on TH and MNCV in animals with SNCI.

Materials and Methods

Animals and treatment

Twenty-four male Wistar rats (200–225 g), procured from the National Toxicology Centre, Pune, India, were used for the present study. These animals were housed under standard animal housing conditions (24 ± 2°C, 70% relative humidity, 12:12 light : dark cycle) with free access to food and water, except during surgery and experiments. The rats were divided randomly into four groups. The MVE group was treated with VEA (50 mg/kg, *ip*) followed by MCA (500 µg/kg, *ip*). Treatment was initiated 2 days post-surgery, with the control, placebo and groups of animals treated only with vehicle UNTR . TH was assessed on day 0 (just before surgery) and day 2 (before MVE/vehicle treatment). On the 15th day after surgery, the animals were tested for TH followed by MNCV. All experiments were carried out between 09:00–17:00 h and were then followed by the drug/vehicle treatment.

All procedures were reviewed and approved by the Institutional Animal Ethics Committee of the Poona College of Pharmacy, Pune, India. The experiments were performed according to the ethical guidelines mentioned by Zimmermann (1986) [32].

Drugs

MCA and pentobarbital sodium (a gift from Emcure Pharmaceuticals, Pune, India) were dissolved in physiological saline, and VEA (Hi Media, Mumbai, India) was suspended in 1% Tween 80 (Loba Chemie, Mumbai, India). The *ip* injections were 1 ml/kg in volume. All drug weights refer to the salt form.

Surgery

Under deep anesthesia produced by pentobarbital sodium (50 mg/kg, *ip*), 12 rats received a unilateral sciatic nerve crush injury [7]. The right sciatic nerve was carefully exposed at the right gluteal region and the crush injury was performed with a jeweler forceps (No. 5) for three successive periods of 10 s. The incision site was then closed. All surgical procedures were performed under sterile operating conditions. Six rats from this group were treated with vehicle (UNTR group) while the other six rats received MVE. In the placebo group of animals (n = 6), the right sciatic nerve was exposed but was left intact. Both the control (n = 6) and placebo groups of animals were treated with vehicle. Treatments for all groups of animals began from day 2 and continued until day 15 after surgery [18, 19, 29].

Thermal hyperalgesia (TH)

TH was assessed using an Ugo Basile Hot Plate Analgesiometer (Versace, Italy). The hot plate was maintained at 55 ± 0.1°C and the paw withdrawal latency (PWL) was performed to assess the number of seconds required for reaction to the thermal stimulus. A cut-off time of 22 s was set to avoid tissue damage. The test was repeated three times at an interval of 15 min, and each test was carried out by a different blind observer. The final response was reported as an average of the three tests.

Motor nerve conduction velocity (MNCV)

Rats were anesthetized using pentobarbital sodium (50 mg/kg, *ip*). The body temperature of the animals was maintained at 37°C under a heating lamp. The right sciatic and tibial nerves were stimulated at the sciatic and tibial notches, respectively, by a 0.1-ms square wave pulse delivered through a pair of monopolar needle electrodes (1.0–1.5 mA, 2.0 mV/D). Responses were recorded from the indigital plantar muscles using a student's biopac data acquisition system (Santa Barbara, CA, USA). MNCV was determined by measuring the distance between sciatic and tibial nerve stimulation, and the data were immediately recorded and printed. All calculations were verified by a blinded investigator.

Statistical analysis

For both TH and MNCV, data were expressed as the mean ± SEM for 6 animals in each group. Statistical significance between groups was determined by one-way ANOVA followed by Tukey *post-hoc* comparisons. Data were considered significant at $p < 0.001$.

Results and Discussion

Our study demonstrated that early co-administration of VEA and MCA after the introduction of sciatic nerve crush lesions ameliorates neuropathic pain behavior by improving PWL and MNCV in rats.

Previous studies have shown that mechanical injury to the peripheral nerves triggers numerous biochemical events due to endothelial damage. This leads to an inflammatory response followed by an increased vascular permeability and edema. The resulting increase in endoneural fluid pressure releases various endogenous chemical mediators from neutrophils. These mediators, in turn, produce ROS and cytokines. The increased oxidative stress then further aggravates the nerve damage [3, 5, 13, 16, 17, 34].

The behavioral symptoms, which develop simultaneously, include hyperalgesia, spontaneous pain, abnormal gait and weight bearing on the opposite side of the operated limb [4, 22]. These symptoms have been reported to progress within 1–2 days post nerve insult [6, 22]. We observed similar symptoms in animals with crush lesions on day 2 [18, 19, present study] after surgery. Their operated leg was flexed backward, a reflex that was absent in the placebo group. In addition, a decrease in PWL to thermal stimulus was observed in the UNTR (6.2 ± 0.23 s) and MVE (6.5 ± 0.27 s) groups of animals, and this effect was not seen in the placebo group (12.27 ± 0.41 s, Tab. 1). These findings confirmed the presence of neuropathic pain due to SNCI [18, 19, 22].

Tab. 1. Effect of MVE on TH produced by SNCI in rats (latency in seconds)

	Day 0	Day 2	Day 15
Control	12.88 ± 0.69	13.02 ± 0.41	13.85 ± 0.70
Placebo	13.22 ± 0.62	12.27 ± 0.413	12.93 ± 0.59
UNTR	13.5 ± 0.43	6.32 ± 0.23*	6.33 ± 0.3*
MVE	12.08 ± 0.69	6.5 ± 0.27*	13.53 ± 0.5

Paw withdrawal latency (± SEM) was observed for control, placebo, UNTR and MVE groups of animals on days 0, 2 and 15 (n = 6 for each group). On day 2, a significant decrease in PWL was observed in UNTR and MVE-treated groups as compared to placebo [$p < 0.001$, $F(3,20) = 112.9$]. Fifteen days post-surgery, PWL improved in MVE-treated animals, which was comparable to placebo. Tukey *post-hoc* tests revealed a significant decrease in PWL in the UNTR group indicating TH [$p < 0.001$, $F(3,20) = 43.73$]. Data compared with placebo; * $p < 0.001$

Fifteen days post-surgery, a significant reduction in TH was observed for the UNTR (6.33 ± 0.3 s) group of animals, indicating the presence of neuropathic pain. Furthermore, the PWL was observed to improve in MVE (13.53 ± 0.5 s)-treated animals. This improvement in PWL was comparable to placebo-treated animals (12.93 ± 0.6 s), indicating recovery from TH induced by nerve crush injuries (Tab. 1).

The endoneural edema was found to increase intraneural pressure followed by a decrease in nerve blood flow. If uncorrected, this improper circulation leads to ischemia, which develops into Wallerian-like axonal degeneration [13, 33]. This phenomenon may account for the observed decrease in nerve conduction velocity [24]. We observed a significant reduction in MNCV for the UNTR (22.32 ± 6 m/s) group of animals at 15 days post-surgery. However, there was no significant difference in MNCV for placebo (49.98 ± 3.0 m/s) and MVE (41.14 ± 4.35 m/s)-treated animals, indicating a possible recovery of the crushed nerve in the MVE-treated group (Fig. 1).

Past studies have shown that both VE [18, 24] and MCA [19, 28] supplementation improved nerve func-

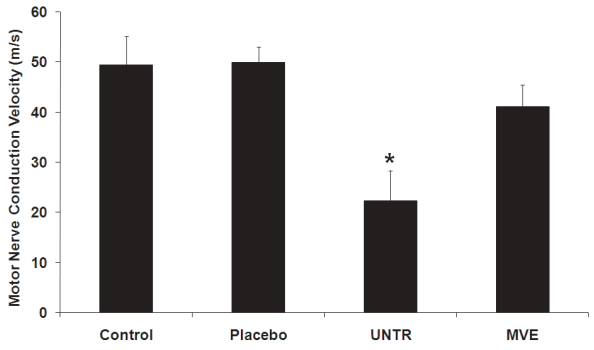

Fig. 1. MNCV (± SEM), measured on day 15 for control, placebo, UNTR and MVE-treated animals. A reduction in conduction velocity in the UNTR group of animals was observed. Early MVE treatment improved the conduction velocity, as there was no significant difference in MNCV between placebo and MVE-treated animals [$p < 0.001$, $F(3,20) = 21.23$]. The number in parentheses represents sample size for each group. Data compared with placebo. * $p < 0.001$

tion in experimental neuropathies. In addition, from our earlier observations, animals exposed to MCA [19] showed a quick onset of recovery from TH, as compared to animals treated with VEA alone [18], in surgery-induced neuropathies. Although MNCV was recovered by day 15 for both VEA [18] and MCA [19] treated animals, complete recovery was not observed. Hence, we hypothesized that an early exposure to both VEA and MCA immediately after the development of pain symptoms might prevent the progression and development of neuropathic pain. In the present study, administration of MVE led to a complete recovery from TH and significantly improved MNCV 15 days post-surgery. MCA, which is a powerful neuroprotective agent, might facilitate early nerve recovery. This effect of MCA could be attributed to its ability to regenerate nerve fibers [21, 30, 31]. Additionally, nerve VE levels were found to decrease following SNCI [3, 27]. Therefore, a decrease in oxidative stress followed by VE supplementation might shorten the recovery time of the injured nerves. These results show a promising neuroprotective effect of MVE co-administration after nerve crush injury. However, further investigations are required to understand the underlying mechanisms by which these agents act.

Acknowledgments:
ASM was supported by a Junior Research Fellowship from AICTE, New Delhi. MCA and pentobarbital sodium were generously provided by Emcure Pharmaceuticals, Pune. The authors are thankful for the technical assistance of Mr. Amol Muthal, Mr. Sachin Arora and Mr. Ganshyam Shrivastav.

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Received:
July 30, 2009; in revised form: October 1, 2009.