eISSN 1303-1775 • pISSN 1303-1783

Original Article

Neuroprotective effect of vitamin E acetate in models of mononeuropathy in rats

Published online 19 May, 2008 © http://www.neuroanatomy.org

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Received 6 September 2007; accepted 8 May 2008

ABSTRACT

Direct injury to a peripheral nerve causes an increase in free oxygen radicals, which can lead to tissue damage. Vitamin E is a major antioxidant used clinically and its study in nerve injury models has not been encountered in the literature. The objective was to study the neuroprotective actions of vitamin E acetate 50 mg/kg by using partial sciatic nerve ligation and sciatic nerve crush injury models in wistar rats. The parameters used were thermal hyperalgesia, motor function test and motor nerve conduction velocity. A steady improvement in thermal hyperalgesia was seen in vitamin E treated animals on day 8th (7.96±0.18 s) and day 7th (8.26±0.15 s) in the models of partial sciatic nerve ligation and sciatic nerve crush injury respectively. There was a reduction in pain which was observed behaviorally in motor function test in both the models and also was observed an improvement in motor nerve conduction velocity of vitamin E treated animals which steadily increased on 15th day (31.59±1.41 m/s) and 30th day (39.29±2.07 m/s). These findings indicate that vitamin E acetate has a promising neuroprotective action in treating hyperalgesia and also improving conduction velocity in the model of nerve ligation and nerve crush injury in rats. © **Neuroanatomy. 2008; 7: 33–37.**

Key words [vitamin E acetate] [partial sciatic nerve ligation] [sciatic nerve crush injury] [thermal hyperalgesia] [motor nerve conduction velocity]

Introduction

It is now very well known that events like nerve ligation directly causes mechanical injury to the nerve and symptoms produced in such animals resemble the symptoms produced in causalgia in humans. The animal model mimics both hyperalgesia (increased response to noxious stimuli) and allodynia (pain response to low threshold stimulus) [1–4]. Furthermore, it is also reported that extent of nerve trapped in the ligature also affects the effects elicited post surgery and most importantly the pain so produced post operatively is like burning sensation similar to burning sensation in causalgic humans [3,5,6].

Direct mechanical injury or ischemia or both can cause acute endothelial injury that can result in endothelial edema, agranulocyte plug or microvascular thrombosis. These factors interrupt the reflow and can cause continuous fiber injury. Moreover, endoneural edema may develop due to microvascular compression. Toxic substances released from neutrophils and macrophages after injury can impair tissue protection in normal conditions and permit the accumulation of free oxygen radicals which increase tissue destruction and cause tissue damage [5]. Reactive oxidant species (ROS) are critically involved in the development and maintenance of neuropathic pain. Studies suggest that systemic administration of non-toxic doses of free radical scavengers could be useful for treatment of neuropathic pain [7]. Also deficiency of some vitamins in the diet could lead to neuropathic pain [8].

Vitamin E (VE) is considered the most effective liposolouble antioxidant found in the human biological system. It interacts with free radicals and prevents lipid peroxidation [9,10]. Clinically, VE supplementation led to electrophysiological recovery of sensory conduction and evoked potentials [11]. Also VE supplementation in cancer patients showed that VE may have an important neuroprotective effects [12].

The objective of the study was to evaluate anti-hyperalgesic and neuroprotective effect of vitamin E acetate (50 mg/kg, O.D.) after sciatic nerve lesion produced by partial sciatic nerve ligation and sciatic nerve crush injury in rats.

Material and Methods

Animals

All the experimental procedure used in this study were reviewed and approved by Institutional Animal Ethical Committee of Poona College of Pharmacy, Pune, India [13]. Forty eight adult female Wistar rats (175-225 g) were obtained from National Toxicology Centre, Pune, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A 12:12 light:dark cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet.

Mononeuropathy caused by Partial Sciatic Nerve Ligation (PSNL) in rats

The method of Seltzer et al [14] was followed. The animals were divided into control (vehicle treated), sham (vehicle treated), untreated and VE treated groups.

Preparation of animals

Under ether anesthesia and aseptic conditions the right sciatic nerve was exposed at high thigh level. In sham operated animals the nerve was left intact and the wound was closed with 2 muscle sutures and 3-4 skin sutures.

In experimental animals the sciatic nerve underwent partial injury. The dorsum of the nerve was carefully freed from surrounding connective tissues at a site near the trochanter just distal to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve. Using an iris forceps the nerve was fixed in its place by pinching the epineurium on its dorsal aspect, taking care not to press the nerve against underlying structures. A 4-0 silk suture was inserted into the nerve with 3/8 curved, reverse-cutting mini needle, and tightly ligated so that the dorsal 1/3 – 1/2 of the nerve thickness was trapped in the ligature. The wound was then closed as in sham operated rats. In all rats the left leg and sciatic nerve were untouched. The animals were allowed to recover after surgery. From day 2 onwards VE (50 mg/kg) once daily, O.D. was administered and continued for 30 days. The observations were recorded daily in the morning (between 10 am to 12 pm) and doses were administrated immediately afterwards.

The effect of VE was studied on the following parameters:

Thermal hyperalgesia (TH)

TH was assessed using Ugo Basile Hot Plate Analgesiometer (at 55±1°C) [15]. Antinociceptive effects were determined according to the latency (in seconds) of limb withdrawal to the noxious thermal stimulation. Cut off value of planter test was set to 22 s to prevent limb injury. The pain thresholds were tested on day 0, 2, 4, 6, 8, 10, 12, 14, 21 and 28.

Motor function test (MFT)

Motor function was monitored by observation of spontaneous gait and hind paw posture [16]. Each animal was placed in a plastic box with plexiglass walls and allowed to habituate for at least 5 minutes before the observation period. One animal at a time was observed for 15 min (3 x 300 s). Different positions of the lesioned hind paw were rated according to a numerical scale described by Attal et al [16]. The observations were made on day 0, 2, 15 and 30.

Mononeuropathy caused by Sciatic Nerve Crush Injury (SNCI) in rats

The method of Bishofs et al [17] was followed. The animals were divided into control (vehicle treated), sham (vehicle treated), untreated, and VE treated groups.

Preparation of animals

Under ether anesthesia and aseptic conditions the right sciatic nerve was exposed at high thigh level. In sham operated animals the nerve was left intact and the wound was closed with 2 muscle sutures and 3-4 skin sutures.

In experimental animals the sciatic nerve underwent crush injury. The dorsum of the nerve was carefully freed from surrounding connective tissues at a site near the trochanter just distal to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve. Using an iris forcep the nerve was fixed in its place by pinching the epineurium on its dorsal aspect, taking care not to press the nerve against underlying structures. A blunt forcep was used to crush the nerve twice for a period of 30 s with an interval of 60 s in between. The wound was then closed as in sham operated rats. In all rats the left leg and sciatic nerve were untouched. The animals were allowed to recover after surgery. From day 2 onwards VE (50 mg/kg) treatment once daily, O.D. was started and continued for 30 days.

The effect of VE was studied on the following parameters:

Thermal hyperalgesia (TH)

TH was assessed as described earlier on day 0, 2, 7, 14, 21, and 28.

Motor function test (MFT)

MFT was assessed as described earlier on day 0, 2, 15, and 30.

Motor Nerve Conduction Velocity (MNCV)

The experiment was performed on the same group of rats on day 15th and 30th day. Rats were anesthetized using thiopental sodium (50 mg/kg, i.p.) for electrophysiological recording. MNCV was recorded by stimulating the sciatic and tibial nerves at sciatic and tibial notch 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) through a stimulator. Responses were recorded from the indigital plantar muscles using Students Biopac data acquisition system (Santa Barbara, CA, USA).

Statistical Analysis

Data was expressed as mean ± SEM of 6 animals in each group. To determine the statistical significance, ANOVA followed by Tukey-Kramer test (Instat/Graphpad) was used. Differences between means were considered statistically significant if $p<0.001$. For motor function test Kruskal-Wallis test followed by Dunn was used.

Results

Effect of vitamin E acetate (50 mg/kg) on thermal hyperalgesia in Partial Sciatic Nerve Ligation (PSNL) model in rats

The latency (sec) of thermal hyperalgesia did not change significantly in normal animals. On the other hand, animals of untreated group showed a significant decrease in latency from day 2 (3.24±0.13 s) to day 28 (3.52±0.21 s) indicating feeling of pain when exposed to thermal stimuli. In the sham treated group, there was decrease in latency on day 2 (7.67±0.32 s) only. Later on the animals did not exhibit decrease in latency. VE treatment showed improvement in the latency from day 8th onwards (7.96±0.18 s). Maximum reduction in latency was observed during day 4 (5.9±0.1 s) and day 6

Table 1. Effect of vitamin E acetate (50 mg/kg) (VE) on Thermal hyperalgesia in Partial sciatic nerve ligation (PSNL) model in rats (Latency in seconds).

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 21	Day 28
Normal	10.01±0.31	10.36±0.42	10.5±0.24	10.0±0.32	9.61±0.16	10.72±0.08	10.68±0.16	10.65±0.16	10.98±0.09	10.21±0.12
Sham	9.23±0.24	7.67±0.32	10.0±0.26	9.65±0.17	9.03±0.18	10.25±0.14	10.48±0.14	10.54±0.25	10.61±0.08	10.43±0.11
Untreated	10.4±0.26	3.24±0.13	3.2±0.09	3.55±0.12	3.64±0.12	3.28±0.09	3.82±0.13	3.81±0.16	3.21±0.09	3.52±0.21
VE	9.51±0.2	3.24±0.09	5.9±0.1	7.12±0.24	7.96±0.18	8.66*±0.16	9.82*±0.12	10.02*±0.24	10.24*±0.09	10.19*±0.34

*Data expressed in Mean ± S.E.M. ANOVA followed by Tukey test. p <0.001 = *. Data of VE group was compared with that of untreated group. n=6*

Table 2. Effect of vitamin E acetate (50 mg/kg) (VE) on Motor function test in Partial sciatic nerve ligation (PSNL) model in rats (Motor function test scores).

	Day 0	Day 2	Day 15	Day 30
Normal	0.0	0.0	0.0	0.0
Sham	0.0	0.271±0.05	0.087±0.027	0.099±0.02
Untreated	0.0	2.92±0.08	2.98±0.09	3.43±0.8
VE	0.0	2.77±0.09	1.26*±0.04	1.14*±0.09

*Data expressed in Mean ± S.E.M. Kruskal Wallis followed by Dunn. p <0.001 = *. Data of VE group was compared with that of untreated group. n=6*

Table 3. Effect of vitamin E acetate (50 mg/kg) (VE) on Thermal hyperalgesia in Sciatic nerve crush injury (SNCI) model in rats (Latency in seconds).

	Day 0	Day 2	Day 7	Day 14	Day 21	Day 28
Normal	11.87±0.48	11.6±0.49	11.3±0.307	10.95±0.33	10.98±0.31	11.48±0.20
Sham	11.91±0.32	9.95±0.22	10.75±0.23	11.4±0.37	11.3±0.20	11.38±0.34
Untreated	11.92±0.20	4.97±0.19	5.26±0.11	5.23±0.06	4.95±0.09	4.78±0.19
VE	11.92±0.2	4.9±0.14	8.26±0.15	9.21*±0.19	11.01*±0.34	11.63*±0.19

*Data expressed in Mean ± S.E.M. ANOVA followed by Tukey test. p <0.001 = *. Data of VE group was compared with that of untreated group. n=6.*

Table 4. Effect of vitamin E acetate (50mg/kg) (VE) on Motor function test in Sciatic nerve crush injury (SNCI) model in rats (Motor function test scores).

	Day 0	Day 2	Day 15	Day 30
Normal	0.0	0.0	0.0	0.0
Sham	0.0	0.271±0.05	0.087±0.027	0.099±0.02
Untreated	0.0	2.92±0.08	3.16±0.09	3.03±0.074
VE	0.0	2.7±0.13	1.29*±0.07	1.21*±0.05

*Data expressed in Mean ± S.E.M. Kruskal Wallis followed by dunn. p <0.001 = *. Data of VE group was compared with that of untreated group. n=6*

Table 5. Effect of vitamin E acetate (50mg/kg) (VE) on Motor nerve conduction velocity in Sciatic nerve crush injury (SNCI) model in rats (Conduction velocity in m/s).

	15th day MNCV	30th day MNCV
Normal	52.75±2.68	53.01±3.99
Sham	47.19±3.85	47.86±2.59
Untreated	15.29±0.71	13.87±0.58
VE	31.59*±1.41	39.29*±2.07

*Data expressed in Mean ± S.E.M. ANOVA followed by Tukey test. p <0.001 = *. Data of VE group was compared with that of untreated group. n=6*

(7.12±0.24 s) and appeared to be due to gradual recovery after administration of VE from day 2 (3.24±0.09 s). Non-significant change in latency observed from day 10–day 28 (10.19±0.34 s) indicate beneficial effect of VE due to complete recovery from thermal algesia (Table 1).

Effect of vitamin E acetate (50 mg/kg) on Motor function test in Partial Sciatic Nerve Ligation (PSNL) model in rats

MFT scores were 0 in all four groups on day 0. In the normal group these scores were not changed on subsequent days of observation. In the sham treated groups, the scores observed were less compared to that of VE or untreated group. The untreated group showed increase in the progressive scores from day 2 (2.92±0.08) to day 30 (3.43±0.8) indicating the presence of pain in the animals. On the other hand in the VE treated group, significant reduction in MFT score was observed on day 15 (1.26±0.05) and day 30 (1.14±0.09), indicating a gradual amelioration of neuropathic pain (Table 2).

Effect of vitamin E acetate (50 mg/kg) on Thermal hyperalgesia in Sciatic Nerve Crush Injury (SNCI) model in rats

In normal animals, the pain latency did not show any remarkable change during the observation period of day 0 to day 28. In sham treated group reduction in pain latency or thermal hyperalgesia was observed on day 2 (9.95±0.22 s) but not on subsequent days. The untreated group showed significant reduction of latency from day 2 (4.97±0.19 s) to day 28 (4.78±0.19 s) indicating presence of pain. In VE treated group, on day 2 (4.9±0.14

s) significant reduction of latency was observed which gradually increased on day 7 (8.26±0.15 s) till day 28 (11.63±0.19 s), indicating recovery from algesia induced by heat (Table 3).

Effect of vitamin E acetate (50 mg/kg) on motor function test in Sciatic Nerve Crush Injury (SNCI) model in rats

MFT scores were 0 in all four groups on day 0. In the normal group these scores were not changed on subsequent days of observation. In the sham treated groups, the scores observed were less compared to that of VE or untreated group. The untreated group showed increase in the progressive scores from day 2 (2.92±0.08) to day 30 (3.03±0.07) indicating the presence of pain in the animals. On the other hand in the VE treated group, significant reduction in MFT score was observed on day 15 (1.29±0.07) and day 30 (1.21±0.05), indicating a gradual amelioration of neuropathic pain (Table 4).

Effect of vitamin E acetate (50mg/kg) on motor nerve conduction velocity in Sciatic Nerve Crush Injury (SNCI) model in rats

The MNCV in the normal animals as well as sham treated animals did not significantly change on day 30 (53.01±3.99 m/s, 47.86±2.59 m/s respectively) as compared to day 15 (52.75±2.68 m/s, 47.19±3.85 m/s respectively). The untreated group showed reduction in MNCV on day 30 (13.87±0.58 m/s) as compared to day 15 (15.29±0.71 m/s) indicating loss of nerve function due to crush injury. In VE treated group the conduction velocity recorded on day 30 (39.29±2.07 m/s) was more than that on day 15 (31.59±1.41 m/s) indicating improved conduction due to drug treatment (Table 5).

Discussion

Patients with peripheral nerve injuries occasionally experience chronic pain. This phenomenon is classified as neuropathic pain. Recently several animal models of neuropathic pain have been evaluated and there appear to be some similarities between these models and the clinical features of human patients [9, 18]. The majority of currently used neuropathic pain models share alterations in hind limb cutaneous sensory thresholds following partial injury to a peripheral (usually sciatic) nerve as a common feature. In particular, demonstration of hyperalgesia to noxious thermal stimuli and allodynia to cold and mechanical stimuli are used as outcome measures. Three most commonly used models are the chronic constriction injury (CCI) of sciatic nerves, the partial sciatic nerve ligation model (PSNL), and the spinal nerve ligation model (SNL) [4]. Nerve crush might be considered an extreme version of compressive nerve injury with an enhanced degree of vasa nervorum disruption [19]. Three factors are thought to cause hyperalgesia in the sciatic nerve ligation model, first is the ectopic discharge generated from injured axons, second is release of cytokines from the inflammatory cells around the injured nerve and third is plastic changes in the sensory pathways to the spinal cord and brain [18].

There is evidence that severe peripheral nerve ischemia from vascular ligation damages endothelium, resulting in swelling, luminal narrowing and no re-flow. Although compression may result in temporary circulatory arrest it is unclear wheather this insult permanently injures nerve micro vessels. Comprehensive ischemia if maintained long enough, might induce no re-flow, as in the ligation experiments [19].

In a serious trauma like crush, a short period of localized total or subtotal ischemia is followed by evident increase in endoneural fluid pressure and impairment of the normal capillary blood flow in the endoneurium. These events results in the release of the endogenous chemical mediators, increase in vascular permeability and impairment of blood nerve barrier. Endothelial and intraneural edema with inflammatory response follows this process [5]. The peripheral nerve responds to trauma by an inflammatory reaction with increased vascular permeability and intraneural edema, local ischemia in the tissue causes metabolic impairment, which in turn allows the production of the toxic oxygen metabolites such as superoxide anion, hydrogen peroxide and hydroxyl radicals by the polymorphoneuclear leucocytes that infiltrate the lesion site. Free radicals and cytokines which are responsible for cell damage are released from neutrophills [6].

VE is considered as one of the principle protective mechanism against oxidative damage in neuronal tissue. VE is the major lipid soluble chain breaking antioxidant in the body tissues and effectively protects against neuronal damage [10,20]. VE is capable of indirectly participate in the reduction of oxidative stress in diabetic patients by its antioxidant activity [21]. Experimental studies have shown that the use of VE after ischemia/reperfusion injury in animals not only attenuated the oxidative injury of the muscle cells but also reduced the formation of edema in these cells, which means that they have partial protective action [9]. VE has protective effects o the retina during retinal ischemia-reperfusion injury [22]. Also suplementation of patients receiving cisplatin chemotherapy with VE decresead the incidence and severity of peripheral neurotoxicity [12].

Results of the present study indicated that in PSNL and SNCI models of mononeuropathy, VE (50 mg/kg, O.D., 30 days) gradually reduced the pain latency (sec) produced by thermal stimulus (Table 1, 3). The onset was observed after 8 days of treatment in PSNL and after 7 days in SNCI model. Further continuation of treatment resulted in restoration of latency to heat stimuli to pre surgery period. The present study was further extrapolated to MFT, a behavioral parameter of assessing mononeuropathy in PSNL and SNCI. VE (50 mg/kg, O.D., 30 days) treatment improved stance and pain endurance caused by nerve injury (Table 2, 4). After 15 days of treatment the pain scale was not significantly different compared to that of day 0, indicating amelioration in pain. Reduction in MNCV is an indication of neuropathic pain, while restoration of passage of impulse indicate an increase in conduction velocity. MNCV in untreated group was reduced while in VE (50 mg/kg, O.D., 30 days) treated group increase in the conduction velocity was observed (Table 5).

Conclusion

Our investigation suggest that vitamin E acetate (50 mg/kg, O.D., 30 days) is effective against TH in PSNL and SNCI models of mononeuropathy in rats. The results conclude that vitamin E acetate can be used in improving nerve conduction velocity which opens a possibility of exploring the potential of vitamin E acetate (50 mg/kg) in the treatment of peripheral neuropathy.

Acknowledgements

We thank Dr. S. S.Kadam and Dr. K. R. Mahadik (Bharati Vidyapeeth University, Poona College of Pharmacy, Pune) for their constant encouragement.

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