Print ISSN 2319-2003 | Online ISSN 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

doi: 10.5455/2319-2003.ijbcp20140621

Research Article

Protective role of vitamin E on drug-induced neuropathy

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Received: 13 April 2014
Accepted: 27 April 2014

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ABSTRACT

Background: The objective of this study was to look into the protective role of vitamin E (Vit.E) on drug-induced neuropathy.
Methods: The study involved 18 albino rats; rats were divided into 3 Groups; Group 1 - control (n = 6), Group 2 - anti-leukemic drugs treated rats (n = 6), Group 3 - anti-leukemic drugs and Vit.E treated rats (n = 6). Anti-leukemic drugs which included vincristine (VCR), L-asparaginase (L-Asp), doxorubicin (ADR), prednisolone (PDN), were administered to Group 2 and Group 3 rats according to acute lymphoblastic leukemia treatment regimen (MCP841). Group 3 rats were given in addition to the anti-leukemic drugs, Vit.E (100 mg/kg bodyweight/orally) daily. Tests for neuropathy were done using tail clip method, tail flick method, hot plate method on the 2nd week and tail clip method on 4th week of therapy.
Results: At the end of 2nd week by tail clip method and tail flick method the mean reaction time of the anti-leukemic drugs alone treated group (Group 2) was increased showing the development of neuropathy. The mean reaction time of the anti-leukemic drugs + Vit.E treated group (Group 3) showed a reduction in the reaction time, showing the protective role of Vitamin E. Hot plate method done at the end of 2nd week showed a decrease in mean reaction time in Group 2 rats compared with Group 3. This could be due to the hyperthermalgesia by VCR. Group 3 was protected by Vit.E.
Conclusion: Observations showed a protective role of Vit.E on drug induced neuropathy.

Keywords: Antioxidants, Anti-leukemic drugs, Vitamin E, Hyperthermalgesia, MCP841 protocol

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. It accounts for one-fourth of all childhood cancers and 75% of all cases of childhood leukemia.[1,2] Modern ALL treatment regimens divide therapy into four main treatment elements viz.; remission induction, central nervous system (CNS) preventive therapy, consolidation and maintenance therapy. Remission induction involves weekly doses of vincristine (VCR), anthracyclines, L-asparaginase (L-Asp) and daily prednisolone (PDN) for 4-6 weeks. CNS preventive therapy includes weekly intrathecal Methotrexate and cranial irradiation. For the study to be specific, the regimen chosen was MCP841.[3] MCP841protocol is one of the treatment regimen for ALL giving higher rate of remission (Table 1).

Most of the drugs used in cancer treatment have a therapeutic index that approaches unity, exerting toxic effects on both normal and tumor tissue even at optimal dosage.[4] Nonselective mechanism of action and resulting low therapeutic indices of the anticancer drugs means that a high incidence of potentially severe toxicities must be tolerated to administer effective doses of these agents.

VCR a microtubule[5] depolymerizing drug produces peripheral neuropathy in humans, that is accompanied by painful paraesthesia and dysthesia[6] and there is no established therapy for this neuropathy. VCR is the only antineoplastic agent having a dose limiting neurotoxicity.[7] Clinical toxicity of VCR is mainly neurological.[8] Neurosensory toxicity was presented in leukemic patients in the form of numbness in the extremities and hyperalgesia and myalgia during the induction phase of treatment. Aley KO *et al*.[9] have reported that VCR produces hyperalgesia and hyper thermalgesia (increased sensitivity to heat stimulation) during the 2nd week of VCR administration. In some studies, patients with the pharmacological doses of vitamin E (Vit.E)

prevented progression of the neurological abnormalities or caused improvement.

Antioxidants are believed to quench free radicals.[10] Vit.E appears to the first line of defense against peroxidation of polyunsaturated fatty acid contained in cellular and subcellular membrane phospholipids. The phospholipids of mitochondria, endoplasmic reticulum and plasma membrane possess affinities for α-tocopherol[11] and Vit.E appears to concentrate at these sites. The α-tocopherol,[11] acts as antioxidants breaking free radical chain reaction as a result of their ability to transfer a phenolic hydrogen to a peroxyl free radical of a peroxidized polyunsaturated fatty acid.

Objectives

The present study was planned to assess the protective role of Vit.E on drug-induced neuropathy.

Table 1: MCP841 protocol for ALL-induction phase.

Day	1	VCR	PDN		ITM
Day	2		PDN	L-Asp	
Day	3		PDN		
Day	4		PDN	L-Asp	
Day	5		PDN		
Day	6		PDN	L-Asp	
Day	7		PDN		
Day	8	ADR-VCR	PDN	L-Asp	ITM
Day	9		PDN		
Day	10		PDN	L-Asp	
Day	11		PDN		
Day	12		PDN	L-Asp	
Day	13		PDN		
Day	14		PDN	L-Asp	
Day	15	ADR-VCR	PDN		ITM
Day	16		PDN	L-Asp	
Day	17		PDN		
Day	18		PDN	L-Asp	
Day	19		PDN		
Day	20		PDN	L-Asp	ITM
Day	21		PDN		
Day	22	VCR	PDN		
Day	23		PDN		
Day	24		PDN		
Day	25		PDN		
Day	26		PDN		
Day	27		PDN		
Day	28		PDN		
Day	29	ADR-VCR	PDN		

VCR: Vincristine, L-Asp: L-Asparaginase, ADR: Anti-leukemic Doxorubicin, PDN: Prednisolone, ALL: Acute lymphoblastic leukemia

Lack of an experimental animal study of toxicities in animals using the MCP841 regimen inspired us to conduct one in albino rats.

METHODS

Study design

This was a prospective study involving 18 albino rats weighing 150-250 g. This was only a preliminary study involving a small number of animals. The rats were divided into 3 groups of 6 animals each (Table 2), maintaining the group average weight equal. Group 1 control (n = 6), Group 2 anti-leukemic drugs treated rats (n = 6), Group 3 anti-leukemic drugs and Vit.E treated rats (n = 6). Anti-leukemic drugs selected were as per the MCP841 protocol as this study was a part of the clinical study in leukemic (ALL) patients who were on this regimen of therapy. Anti-leukemic drugs included VCR-(1.4 mg/m^2/IP), L-Asp-(6000 u/m^2/IP), doxorubicin-(30 mg/m^2/IP), PDN-(40 mg/m)^2 (Table 3). 2nd group was given anti-leukemic drugs as in the induction phase of treatment of ALL as per MCP841 regimen (Table 1). Group 3 rats were given in addition to the anti-leukemic drugs - Vit.E 100 mg/kg body weight orally daily.[12] Group 1 control was given distilled water 1.48 ml orally daily.

Tests for neuropathy were done on the 2nd and 4th week of therapy on Day 13 and 24 i.e., after 2 and 4 doses of VCR, using physical and thermal stimuli. In the 2nd week, test methods used were Tail clip method (Bianchi and David) Tail flick method (Gujral and Khana) and Hot plate method. Tail clip method was repeated in the 4th week.

Tail clip method

In this method, a bull dog clamp with thin rubber sleeves is applied to the base of the rat's tail for 30 sec. Control rats take continuous efforts to dislodge the clip by biting it. This is taken as the reaction time (drug induced neuropathy make the rats indifferent to the clip) reaction time taken for all the animals taken and mean reaction time of each group calculated[13,14] (Plate 1).

Tail flick response

Rats were held in suitable restrainer with tail protruding out. Tail was cleaned properly to avoid interference with the result. Radiant heat[15] applied over the tail on a single spot with the help of a suitable device. The time taken by the animal to withdraw (flick) the tail was taken as reaction time. Screening was done before the experiment and rats showing reaction time 10 sec or less were taken. The cut-off time was set up 20 sec to avoid any further injury to the tail. Reaction time for all the animals were taken, and mean reaction time of each group calculated[14,16] (Plate 2).

Table 2: Demographical data of animals used.

Parameter	Group 1 (n=6)		Group 2 (n=6)		Group 3 (n=6)	
	Mean	SD	Mean	SD	Mean	SD
Weight (g)	186.667	27.325	186.667	39.883	186.667	41.312
Length (cm)	17.667	4.597	21.167	1.602	21.333	1.966
BSA (m^2)	0.033	0.0036	0.032	0.0046	0.032	0.0047

BSA: Body surface area

Table 3: Dosages of drugs given.

VCR	1.4 mg/m IP
L-Asp	6000 u/m IP
ADR	30 mg/m IP
PDN	40 mg/m IP
Vit.E	100 mg/kg bodyweight PO

VCR: Vincristine, L-Asp: L-Asparaginase, ADR: Anti-leukemic Doxorubicin, PDN: Prednisolone

Figure 1: Tail clip method.

Hot plate method

Rats were placed on a hot plate maintained at 55°C. The reaction time was that between placing the animal on the hot plate and licking of the fore or hind paws or jumping reaction. Screening was done before the experiment and rats showing reaction time 10 sec or less were taken. The cut-off time was set up 20 sec to avoid any further injury to the paws. Paws and hot plate were cleaned for uniform temperature distribution. Reaction time for all the animals were taken, and mean reaction time of each group calculated[14,17] (Plate 3).

Hot plate and Tail flick are two different methods of evaluation of nociception. Tail flick test is predominantly a spinal response and Hot Plate is mostly at supraspinal level.[15]

Statistical method used in all the tests was non parametric technique Kruskal–Wallis-one-way analysis of variance.

RESULTS

Tail clip method - 2nd week mean reaction time (Table 4, Figure 4).

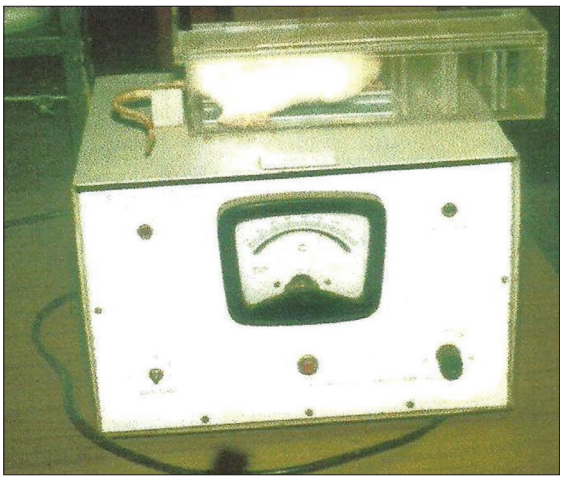

Figure 2: Tail flick method.

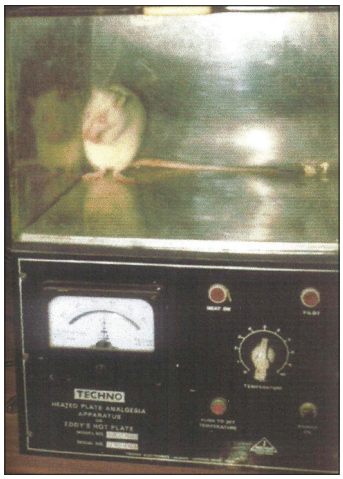

Figure 3: Hot plate method.

There was an increased reaction time in Group 2 compared with Group 3, with a difference of 4.666 sec. $P = 0.442$.

Tail Flick Method-2nd week mean reaction time (Table 5, Figure 5).

There was an increased mean reaction time in Group 2 compared with Group 3, with a difference of 4.500 sec. $P = 0.481$.

Hot plate method-2nd week mean reaction time (Table 6, Figure 6).

There was a decrease in the mean reaction time in Group 2 compared with Group 3, with a difference of 2.250 sec. $P = 0.828$.

Table 4: Tail clip method 2nd week.

Group	Mean reaction time (sec)	SD (sec)	F/H	P
1	4.167	1.169	H=1.633	0.442041
2	9.333	6.028		
3	4.667	2.887		

Values of mean reaction time and standard deviation

Table 5: Tail flick method 2nd week.

Group	Mean reaction time (sec)	SD (sec)	F/H	P
1	8.667	2.309	H=1.464	0.481002
2	14.500	8.062		
3	10	2.828		

Values of mean reaction time and standard deviation

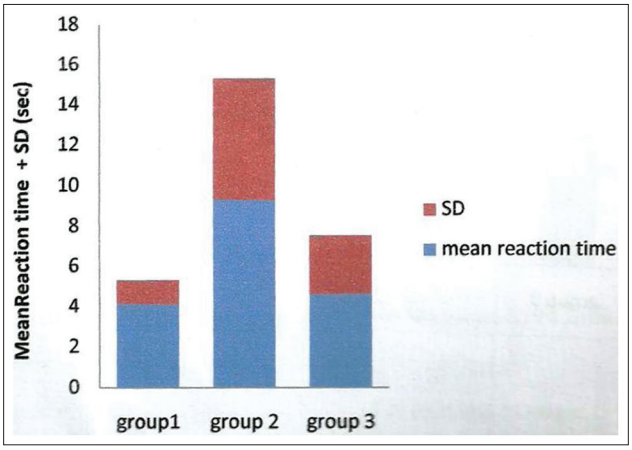

Figure 4: Tail clip method 2nd week (X axis Group 1, Group 2, Group 3. Y axis mean reaction time and standard deviation in seconds of the three groups).

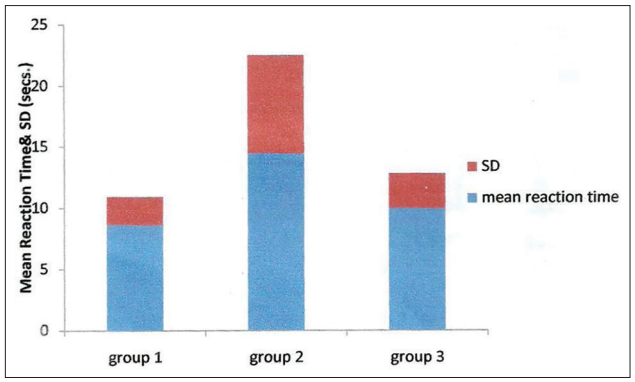

Figure 5: Tail flick method 2nd week (X axis Group 1, Group 2, Group 3. Y axis mean reaction time and standard deviation in seconds of the three groups).

Tail clip method-4th week mean reaction time (Table 7, Figure 7).

There was an increased reaction time in Group 2 compared with Group 3, with a difference of 4.367 sec. $P = 0.222$.

Table 6: Hot plate method 2nd week.

Group	Mean reaction time (sec)	SD (sec)	F/H	P
1	3.625	0.946	H=0.376	0.828640
2	4.417	4.565		
3	6.667	7.062		

Values of mean reaction time and standard deviation

Table 7: Tail clip method 4th week.

Group	Mean reaction time (sec)	SD (sec)	F/H	P
1	6.33	3.215	H=3.006	0.222447
2	12.200	6.017		
3	7.833	7.414		

Values of mean reaction time and standard deviation

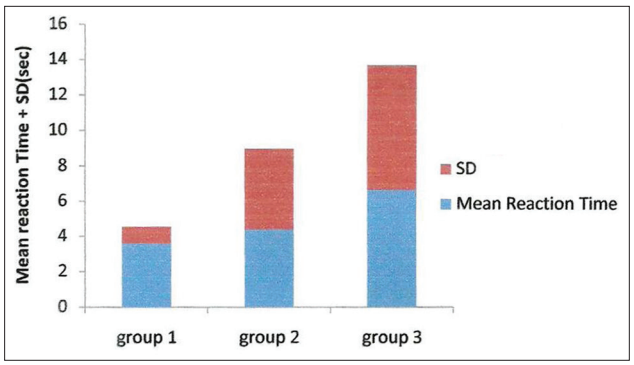

Figure 6: Hot plate method 2nd week (X axis Group 1, Group 2, Group 3. Y axis mean reaction time and standard deviation in seconds of the three groups).

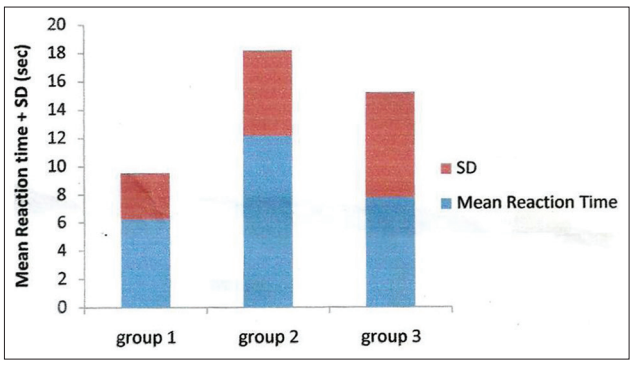

Figure 7: Tail clip method 4th week (X axis Group 1, Group 2, Group 3. Y axis mean reaction.

The mean reaction time of Groups 2 and 3 by tail clip method and tail flick method (using radiant heat)[15] in the 2nd week showed that it was increased in Group 2. 4th week tail clip method also showed an increase in reaction time in Group 2. Even though there was a clinically meaningful difference between Group 2 and Group 3, it was not statistically significant. Because significance was not achieved due to small sample size. Whereas in hot plate method (2nd week) where we used direct heat as the thermal stimulus, we

saw a reduction in reaction time in Group 2, which was anti-leukemic drugs alone treated group. Group 3 showed an increase in the reaction time. This could be due to the hyperthermalgesia produced by VCR which was protected in Group 3 by Vit.E. Here again, *P* was not significant because of the small sample size.

DISCUSSION

The neurologic complication of anti-neoplastic therapy may occur as a result of direct damage to the nervous system (when the agent itself is toxic to the nervous system) or from an indirect damage to the nervous system such as meningitis that occur as a complication of severe myelosuppression from chemotherapy. VCR is the only antineoplastic agent having a dose limiting neurotoxicity. Paresthesia of the hands and feet, loss of tendon reflexes and weakness occur in almost all patients. These effects are usually, but not always reasonable and are more severe in older patients, myalgia, paraesthesias and weakness severe enough to cause foot drops frequently require discontinuation of therapy. The neurologic toxicity of VCR can take many forms, but is most commonly seen as a mixed sensorimotor peripheral neuropathy.

The increase in reaction time noted in the Group 2 might be due to the peripheral neuropathy by drugs especially VCR, which is the well-known neurotoxic agent. Group 3 showing a reduction in reaction time compared to Group 2 might be protected by Vit. E, the antioxidant. Oxidant stress here being the neurotoxic agent VCR.

In acting as antioxidant Vit.E presumably prevents oxidation of essential cellular constituents or prevention of the formation of toxic oxidation products. Vit.E is absorbed from the gastrointestinal tract by a mechanism probably similar to that for the other fat soluble vitamins; bile is essential. Vit.E enter the bloodstream in chylomicrons by way of the lymph. It is taken up in chylomicron remnants by the liver and is secreted in very low density lipoproteins. Subsequently, it becomes associated with plasma β lipoproteins. Vit.E is distributed to all tissues. In its antioxidant role, Vit.E become oxidized. Thereafter, it may be regenerated by other antioxidants particularly ascorbic acid and glutathione.

The reduction in reaction time noted in Group 2 in the 2nd week using a hot plate method where we used direct heat as the thermal stimulus, might be due to the hyperthermalgesia induced by VCR, which was protected by Vit.E in the Group 3.

CONCLUSION

Our findings suggest that Vit.E is likely to be a safe and effective neuroprotectant in patients receiving VCR, and it warrants further experimental animal study in large no. of animals and also clinical evaluation. The induction of neurotoxicity in an animal model and the beneficial effect of prophylactic use of Vit.E as an antioxidant was investigated and confirmed. Implementation of Vit.E administration along with anti-leukemic regimen consisting of VCR would however be possible only after further extensive randomized clinical trials.

ACKNOWLEDGMENTS

Authors are immensely obliged to Mr. S. Muraleedharan Nair, MSc, Medical Statistics, CERTC, Medical College, Trivandrum for lending his specialized help in the statistical analysis.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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doi: 10.5455/2319-2003.ijbcp20140621
Cite this article as: Roy R, Hema CG, Geetha N, Indla R, Chinnathampi T. Protective role of vitamin E on drug induced neuropathy. Int J Basic Clin Pharmacol. 2014;3:523-8.