Original Article

DOI: 10.5582/ddt.2011.v5.3.136

Mechanisms of vincristine-induced neurotoxicity: Possible reversal by erythropoietin

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ABSTRACT: Vincristine (VCR) is a potent anticancer drug, but neurotoxicity is one of its most important dose-limiting toxicities. In this study, we investigated the neurotoxic effect of VCR, the possible mechanisms and the role of erythropoietin (EPO) in the protection against VCR-induced neurotoxicity in a rat model. The neurotoxicity of VCR and protective effect of EPO were examined using the tail flick test and by recording electrophysiological characteristics in isolated sciatic nerve. To elucidate the underlying mechanisms, mRNA expression of *N*-methyl-D-aspartate (NMDA) receptor, an index of glutamate excitotoxicity, and calcitonin gene-related peptide (CGRP), an important regulator of vascular tone, were measured in both spinal cord and sciatic nerves using an RT-PCR method. After intraperitoneal injection at a dose of 150 µg/kg three times weekly for five consecutive weeks, VCR significantly decreased the latency of tail withdrawal reflex, the amplitude of maximum compound action potential (MCAP) and chronaxie, and prolonged the duration of action potential (AP) and relative refractory period (RRP), but it had no effect on conduction velocity. VCR increased NMDA receptor expression and decreased CGRP expression. Forty µg/kg of EPO improved all VCR-induced changes, except chronaxie, while a higher dose of 80 µg/kg reversed all parameters and its effect was more prominent on tail flick test latency and NMDA receptor expression. These results suggested that VCR might cause increased nerve excitability and induce a state of glutamate excitotoxicity through enhancing NMDA receptor expression and diminishing CGRP expression, thus resulting in axonal degeneration. EPO had an obvious neuroprotective effect probably through decreasing NMDA receptor expression and increasing CGRP expression both centrally and peripherally.

Keywords: Vincristine, action potential, *N*-methyl-D-aspartate receptor, Calcitonin gene-related peptide, erythropoietin

1. Introduction

Vincristine (VCR) is a chemotherapeutic agent that can be used in the treatment of many types of human cancer (*1*). It is a purified alkaloid extracted from the periwinkle plant *Vinca rosea* Linn. of the family Apocynaceae (*2*). However, like many chemotherapeutic drugs it is toxic to peripheral nerves, and the development of VCR-induced neuropathy seems to be dose-related and occurs in the early stage of treatment (*2*). Thus, the clinical use of VCR is limited by the predictable development of the neuropathy (*3*). If this effect could be prevented, it might be possible to use VCR more effectively in the treatment of malignant tumors at higher doses and for longer duration. Thus far, the mechanisms of VCR-induced neuropathy are poorly understood, which has hindered the development of protective measures against this toxic effect.

Erythropoietin (EPO), a well-established hematopoietic factor responsible for the production of red blood corpuscles, was discovered to have multiple functions outside the bone marrow. When it was found that recombinant human EPO crosses the blood-brain barrier (*4*), interest has focused on its function in the nervous system. Studies have shown that it reduces injury both centrally in acute ischemic stroke patients (*5*), as well as peripherally in sciatic nerve compression (*6*). Recently, EPO was also demonstrated to protect and reverse experimental diabetic neuropathy (*7*).

Thus, the aim of the present study was to clarify the alterations in VCR-induced peripheral neuropathy using a behavioral assay, the tail flick test, and neurophysiologic studies performed on the rat sciatic nerve. In addition,

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some possible underlying mechanisms of the action of VCR were investigated by measuring *N*-methyl-D-aspartate (NMDA) receptor mRNA and calcitonin gene related peptide (CGRP) mRNA expression in both the spinal cord and sciatic nerves. Furthermore, we studied the potential beneficial role of EPO (at 2 different doses) in preventing VCR-induced peripheral neuropathy and whether it reversed the underlying pathology.

2. Materials and Methods

2.1. *Animals*

Forty male Sprague Dawley rats, weighing between 150-200 g, were purchased from the animal house of the National Research Center, Cairo University. They were given free access to water and food, and maintained on 12 h light/dark cycle. All experimental procedures were carried out in compliance with the guide for care and use of laboratory animals published by the US National Institutes of Health (NIH publication 85-23 revised 1985) and in compliance with the Local Animal Ethics Committee of Kasr Al Aini, Faculty of Medicine, Cairo University.

2.2. *Experimental protocol*

Rats were assigned into the following four equal groups: Group I (Control group), this group of rats received distilled water as a vehicle for 5 consecutive weeks; Group II (VCR-treated group, VCR group), vincristine sulfate (Korea United Pharm. Inc., Chungcheongnam-do, Korea; 1 mg/mL vial) was injected at a dose of 150 µg/kg, intraperitoneally, three times weekly for five consecutive weeks (*8*,*9*); Group III (EPO-treated group 1, EPO1 group), this group of rats was treated with VCR at the same dose and route of administration as in group II and concomitantly received human recombinant EPO (Egyptian International Pharmaceutical Industries Co., 10th of Ramadan City, Egypt; 10,000 IU/mL vial) at a dose of 40 µg/kg, intraperitoneally, three times weekly for five consecutive weeks (*7*); Group IV (EPO-treated group 2, EPO2 group), this group of rats was subjected to the same treatment protocol as in group III, but the EPO dose was doubled (80 µg/kg).

At the end of the experimental protocol, all groups were subjected to the tail flick test as a behavioral nociceptive reaction. Rats were then sacrificed by cervical dislocation followed by decapitation. Sciatic nerves of both limbs were exposed using a longitudinal skin incision in the hind legs. Each nerve was dissected free from the surrounding connective tissue and completely excised with the epineuria intact: One nerve was used for electrophysiological studies and the other one for assessment of gene expression of calcitonin gene-related peptide (CGRP) mRNA and *N*-methyl-D-aspartate (NMDA) receptor mRNA.

2.3. *Electrophysiological recordings*

The sciatic nerve was mounted in a nerve chamber (MLT012/B. AD Instruments) designed for the recording of action potentials from isolated nerves. It contains 15 stainless steel wire electrodes of 0.8 mm diameter spaced at intervals of 5 and 10 mm. Each end of the nerve was tied with short lengths of thread that were pushed into split silicone tubing at either end of the bath to help position the nerve without stretching it. The nerve was positioned over the electrodes and embedded in paraffin oil at 35°C to maximize signal amplitude and prevent drying. To record a monophasic action potential, the sciatic nerve was crushed with a forceps near its distal end.

The proximal part of the nerve was stimulated by 2 platinum stimulating hook electrodes. The recording electrodes were placed 1 to 2 cm apart from the stimulating ones.

The electrophysiological measurements were performed using an AD Instruments (Greenwich, CT) Power Lab 4/25 stimulator and a BioAMP amplifier followed by computer-assisted data analysis (Chart 5.0 and SCOPE 3.7; AD Instruments) and displayed at a sampling rate of 40 K/sec. Sciatic nerves were stimulated with square wave pulses of 0.2 msec duration at 1-10 volts for action potential, conduction velocity and refractory period measurements, and of 10-1,000 µsec at 50 µV-5 V for chronaxie. The parameters were assessed as follows.

2.3.1. *Maximal compound action potential (MCAP) amplitude*

The stimulating voltage was set to produce a maximal compound monophasic action potential using square wave pulses of supra-maximal strength and 0.2 msec in duration. The maximum amplitude was measured from the baseline to the peak of the action potential as shown in Figure 1 (*10*).

2.3.2. *Duration of action potential*

The time elapsed in milliseconds between the onset of depolarization to return to the base line was recorded (*11*).

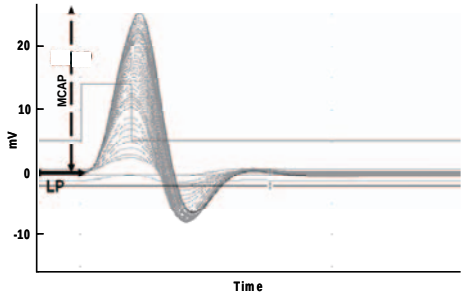

Figure 1. MCAP amplitude profile. MCAP amplitude was measured from the baseline to the peak of action potential. The latent period (LP) is the time elapsed between the application of the stimulus until the start of MCAP.

2.3.3. *Relative refractory period (RRP)*

Twin maximum pulse stimuli with stimulus interval of 2 msec were used to record 2 action potentials. Then, the stimulus interval was decreased gradually by 10 μsec decrements. The interval when the amplitude of the second CAP decreases and reaches 75% of the first maximum CAP was recorded and taken as a standardized measure of refractoriness for comparison between groups (*11*).

2.3.4. *Conduction velocity*

This was measured by dividing the distance between the stimulating and the recording electrodes by the time elapsed between the application of the stimulus until the peak of the MCAP (*12*).

2.3.5. *Chronaxie*

The rheobase was measured as the threshold stimulus voltage for an active response with a long duration pulse. Chronaxie is the pulse width corresponding to twice the rheobase (*12*).

2.4. *Behavioral assay*

As a behavioral test, the tail flick (immersion) test is used to assess the nociceptive response to acute thermal pain stimulus. This measure was chosen because of the role small fiber dorsal root ganglia sensory neurons play in pain transmission (*13*). The animals were restrained in a restrainer cage with their tail hanging free and allowed to adapt for 30 min before testing. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water of exactly 55°C. Within a few seconds, the rat reacted by withdrawing the tail. The latency of this tail withdrawal reflex was recorded using a stop watch in all animal groups (*14*).

2.5. *Detection of NMDA receptor mRNA and CGRP expression mRNA by reverse transcriptase polymerase chain reaction (RT-PCR)*

About 30 mg of nerve and spinal cord tissues were stored at –80°C in lysis buffer containing guanidium thiocyanate and β-mercaptoethanol for RNA extraction.

2.5.1. *RNA extraction*

Total RNA was extracted from both nerve and spinal cord after homogenization according to the manufacturer's instructions. The concentration of extracted RNA was measured spectrophotometrically at 260 nm.

2.5.2. *RT-PCR*

For amplification of the targets, reverse transcription and PCR were run in two separate steps. Briefly, equal amounts of total RNA (6 μg) were heat denatured and reverse transcribed by incubation at 42°C for 90 min with 12.5 U avian myeloblastosis virus reverse transcriptase (AMV) (Promega Corp., Madison, WI, USA), 20 U ribonuclease inhibitor RNAsin (Promega Corp.), 200 nM deoxy-nucleoside 5'-triphosphate mixture, and 1 nM oligo-dT primer in a final volume of 30 μL of 1× avian myeloblastosis virus reverse transcriptase buffer. The reactions were terminated by heating at 97°C for 5 min and cooling on ice. The cDNA samples were amplified in 50 μL of 1× PCR buffer in the presence of 2.5 U Taq DNA polymerase (Promega Corp.), 200 nM deoxy-nucleoside 5'-triphosphate mixture, and the appropriate primer pairs (1 nM of each primer. These sets of primers of CGRP and NMDA were designed from GenBank (accession No. G35510 and 691379, respectively): forward primer, 5'-GAGATC AGGAGTTCAAGACC-3' and 5'-TCCAAACTGGTCA CACCTCACT-3', respectively; reverse primer, 5'-TTGG CTCACTGCAACCTCC-3' and 5'-CAGCTTTGGTGAC AGCATCTCT-3', respectively.

PCR consisted of a first denaturing cycle at 97°C for 5 min, followed by a variable number of cycles of amplification defined by denaturation at 96°C for 1.5 min, annealing for 1.5 min, and extension at 72°C for 3 min. A final extension cycle of 72°C for 15 min was included. Annealing temperature was adjusted to 55°C (*15*).

2.5.3. *Agarose gel electrophoresis*

All PCR products were electrophoresed on 2% agarose stained with ethidium bromide and visualized with a UV transilluminator.

2.5.4. *Semi-quantitative determination of PCR products*

Semi-quantitation was performed using a gel documentation system (BioDO, Analyser) supplied by Biometra. According to the following amplification procedure, relative expression of each gene was calculated following the formula (*15*):

R = Densitometrical units of each gene/Densitometrical units of β-actin

β-actin primers were designed from GenBank (accession No. J00691).

2.6. *Statistics*

All data are expressed as means ± S.E. Statistical analysis was performed using one way ANOVA (Microcal Origin Software, Inc., Version 5) followed by the Tukey test for multiple comparisons. A p value < 0.05 was considered statistically significant.

3. Results

3.1. *Electrophysiological recordings*

3.1.1. *Sciatic nerve MCAP amplitude*

Following VCR injection for 5 weeks, the mean MCAP was significantly reduced compared to the control group ($p < 0.05$) (Figure 2). Concomitant administration of EPO at the lower dose together with VCR in the EPO1 group completely reversed the effect of VCR and no significant difference in the amplitude of MCAP was measured compared to the control group. In the EPO2 group, however, the mean MCAP was significantly lower than in the control group, but still significantly higher than in the VCR group. There was a significantly lower mean MCAP in the EPO2 group compared to the EPO1 group ($p < 0.05$) (Figure 2).

3.1.2. *Duration of action potential*

The action potential duration was significantly prolonged in the VCR-treated group ($p < 0.05$) (Figure 3). In the EPO1 and EPO2 groups, this prolongation was completely abolished and the action potential duration showed no significant change compared to the control group.

3.1.3. *RRP*

With regard to the RRP, it was found to be significantly prolonged in the VCR group compared to the control group ($p < 0.05$) (Figure 4). Again, this effect of VCR was completely abolished by the concomitant administration of the two doses of EPO, so that the RRP in the EPO1 and EPO2 groups was not significantly different from the values of the control group.

3.1.4. *Conduction velocity*

The conduction velocity was not significantly altered in any of the tested groups compared to the control group ($p > 0.05$) (Figure 5).

3.1.5. *Chronaxie*

In the VCR group, chronaxie was significantly shortened

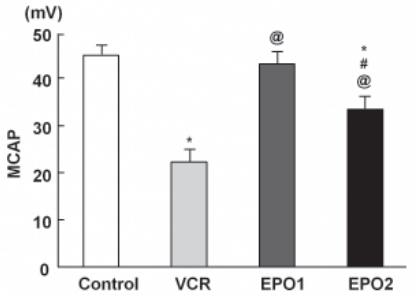

Figure 2. Maximum compound action potential amplitude produced by supramaximal stimulation of the sciatic nerve. Values are expressed as mean ± S.E., * $p < 0.05$ significant *vs.* control, $^{@}p < 0.05$ *vs.* VCR group, $^{\#}p < 0.05$ *vs.* EPO1 group ($n = 10$ rats/group). EPO1 group received 40 μg/kg, three times weekly for five consecutive weeks. EPO2 group received 80 μg/kg, three times weekly for five consecutive weeks.

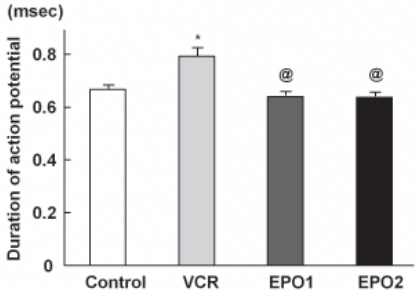

Figure 3. Duration of action potential in VCR, EPO1, and EPO2 groups. Duration of action potential showed a significant prolongation in the VCR group with complete reversal back to control by both doses of EPO. Values are expressed as mean ± S.E., * $p < 0.05$ significant *vs.* control, $^{@}p < 0.05$ *vs.* VCR group, $^{\#}p < 0.05$ *vs.* EPO1 group ($n = 10$ rats/group). EPO1 group received 40 μg/kg, three times weekly for five consecutive weeks. EPO2 group received 80 μg/kg, three times weekly for five consecutive weeks.

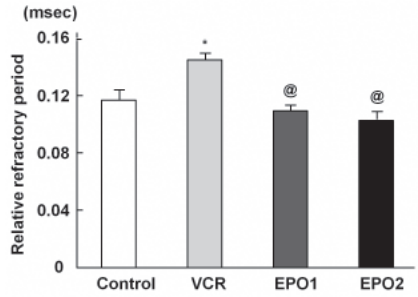

Figure 4. Relative refractory period in VCR, EPO1, and EPO2 groups. RRP was significantly increased after 5 weeks of treatment with VCR and was totally declined with any of the doses used of EPO. Values are expressed as mean ± S.E., * $p < 0.05$ significant *vs.* control, $^{@}p < 0.05$ *vs.* VCR group, $^{\#}p < 0.05$ *vs.* EPO1 group ($n = 10$ rats/group). EPO1 group received 40 μg/kg, three times weekly for five consecutive weeks. EPO2 group received 80 μg/kg, three times weekly for five consecutive weeks.

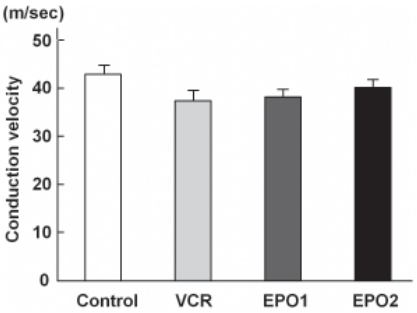

Figure 5. Sciatic nerve conduction velocity in VCR, EPO1, and EPO2 groups. Sciatic nerve conduction velocity measurements showed no significant changes in any of the studied groups. Values are expressed as mean ± S.E. ($n = 10$ rats/group).

Table 1. Results of chronaxie and tail flick test as indices of nerve excitability in the different experimental groups

	Groups*			
	Control	VCR	EPO1	EPO2
Chronaxie (μsec)	89.3 ± 2.0	77.5 ± 3.8[a]	79.2 ± 3.3[a]	92.3 ± 1.2[b,c]
Tail flick test (sec)	14.9 ± 0.6	7.8 ± 0.6[a]	10.6 ± 1.1[a,b]	14.6 ± 0.4[b,c]

* VCR group, vincristine-treated group; EPO1 group, erythropoietin-treated group 1; EPO2 group, erythropoietin-treated group 2. EPO1 and EPO2 groups received 40 μg/kg and 80 μg/kg of EPO, respectively, three times weekly for five consecutive weeks. Values are expressed as mean ± S.E. (n = 10 rats/group). [a] $p < 0.05$ *vs.* control group; [b] $p < 0.05$ *vs.* VCR group; [c] $p < 0.05$ *vs.* EPO1 group.

($p < 0.05$) compared to the control group (Table 1). The lower dose of EPO in the EPO1 group was not able to reverse this effect. The EPO2 group, however, showed a significantly prolonged chronaxie compared to the VCR group ($p < 0.05$), and it was not significantly different from the control (Table 1).

3.2. *Behavioral assay (tail flick test)*

The latency of tail withdrawal reflex in response to immersion of the tail in warm water was significantly shortened in the VCR group compared to the control group ($p < 0.05$) (Table 1). In the EPO1 group, the latency was significantly lower than that of the control group, but significantly higher than in the VCR group ($p < 0.05$) (Table 1). In the EPO2 group, the higher dose of EPO completely reversed the effect of VCR in the tail flick test, so that there was no significant difference between the EPO2 group and control, while the latency was significantly longer than in the VCR and EPO1 groups ($p < 0.05$) (Table 1).

3.3. *NMDA receptor and CGRP gene expression*

Gene expressions of NMDA receptor and CGRP were evaluated using RT-PCR and agarose gel electrophoresis (Figure 6). As shown in Table 2, NMDA receptor expression was significantly increased in the VCR group ($p < 0.05$) in both the spinal cord and sciatic nerves. In the EPO1 group, concomitant administration of EPO at the lower dose with VCR, resulted in a significant decrease in the expression of NMDA receptors compared to the VCR group ($p < 0.05$), but it was still significantly higher than in the control group ($p < 0.05$) in the spinal cord and sciatic nerves. With the higher dose of EPO, the EPO2 group showed a complete reversal of the effect of VCR in the sciatic nerves, as NMDA receptor expression was not significantly different than that of the control group ($p > 0.05$). In the spinal cord, however, the expression of NMDA receptors was significantly lower in the EPO2 group, but it remained higher than in the control group ($p < 0.05$).

With regard to the expression of CGRP, the VCR group showed significantly reduced expression in the spinal cord and sciatic nerves compared to the control group ($p < 0.05$) (Table 2). In the EPO1 and EPO2 groups there was a significant increase of the expression of

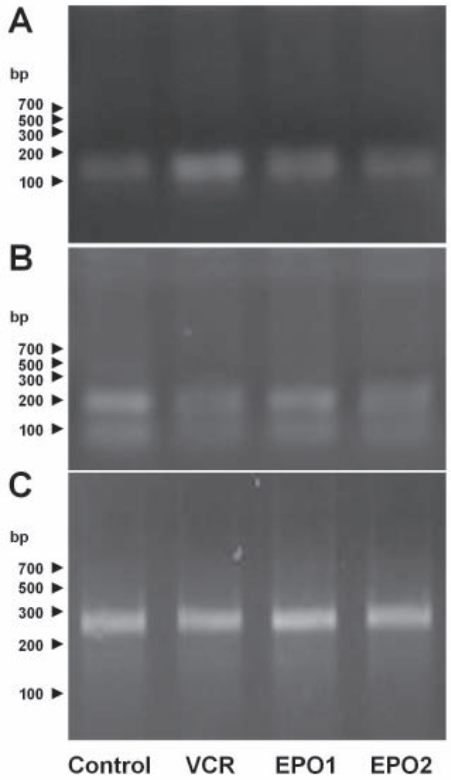

Figure 6. Agarose gel electrophoresis profiles showing PCR products of various genes tested in sciatic nerve. (A) PCR products of NMDA gene (105 bp). **(B)** PCR products of CGRP gene (210 bp). **(C)** PCR products of β-actin gene (256 bp). Lane 1, Control group; Lane 2, VCR group; Lane 3, EPO1 group; Lane 4, EPO2 group.

CGRP in sciatic nerves compared to the VCR group, but it was still significantly lower than in the control group ($p < 0.05$). In the spinal cord, however, CGRP expression in both EPO1 and EPO2 groups was significantly increased compared to the VCR and the control group ($p < 0.05$).

4. Discussion

In the present study, VCR-induced neurotoxicity was tested by electrophysiological measurements. We observed that the amplitude of the MCAP was significantly decreased, while its duration was significantly prolonged in the VCR group. This suggests that a significant number of sensory and motor fibers are affected by the drug as confirmed by reported histological studies, which showed VCR-induced axonal degeneration as well as disorganization of the axonal microtubule cytoskeleton

Table 2. NMDA receptor and CGRP gene expression (in sciatic nerves and spinal cord) in the different experimental groups

	Groups*			
	Control	VCR	EPO1	EPO2
NMDA receptor expression				
In sciatic nerves	1.21 ± 0.09	3.55 ± 0.27[a]	2.19 ± 0.10[a,b]	1.51 ± 0.14[b, c]
In spinal cord	0.02 ± 0.06	1.67 ± 0.21[a]	0.56 ± 0.12[a,b]	0.51 ± 0.03[a,b]
CGRP expression				
In sciatic nerves	4.99 ± 0.29	2.38 ± 0.16[a]	3.91 ± 0.21[a,b]	4.15 ± 0.12[a,b]
In spinal cord	4.63 ± 0.18	1.46 ± 0.19[a]	6.16 ± 0.15[a,b]	6.78 ± 0.26[a,b]

* VCR group, vincristine-treated group; EPO1 group, erythropoietin-treated group 1; EPO2 group, erythropoietin-treated group 2. EPO1 and EPO2 groups received 40 µg/kg and 80 µg/kg of EPO, respectively, three times weekly for five consecutive weeks. Values are expressed as mean ± S.E. (n = 10 rats/group). [a] $p < 0.05$ *vs.* control group; [b] $p < 0.05$ *vs.* VCR group; [c] $p < 0.05$ *vs.* EPO1 group. Abbreviations: NMDA, *N*-methyl-D-aspartate; CGRP, calcitonin gene related peptide.

and increase in the caliber of unmyelinated sensory axons (*9,16*). On the other hand, no significant change was observed in conduction velocity. The preservation of conduction velocity, despite a reduced amplitude and prolonged duration of action potential, may reflect that axonal degeneration may have been too mild and too early to show an effect on nerve conduction and suggests an early stage of myelin disruption.

In the present study, the RRP was found to be significantly prolonged in the VCR group. RRP measurement is more sensitive than routine measures of nerve conduction in detection of axonal disorders (*17*) and early neurotoxicity (*18*). To our knowledge, no other study has investigated the effect of VCR on RRP. However, another study, using Oxaliplatin (a chemotherapeutic drug) observed a prolonged RRP (*19*). It proposed that this might be mediated through an effect on axonal voltage-gated transient Na^+ channels, which is involved in the subsequent process of axonal degeneration.

In this study, VCR induced shortening of chronaxie, suggesting increased nerve excitability. This is further evidenced by the results of the tail-flick test which showed significant shortening of the reaction time, indicating a decreased nociceptive threshold to thermal stimuli. The observed increased excitability suggests the presence of an excitotoxicity. Glutamate mediates excitatory synaptic transmission through the activation of ionotropic glutamate receptors that are sensitive to NMDA, amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), or kainate. Excess and sustained activation of these receptors causes fulminant neuronal death, namely, glutamate excitotoxicity (*20*). The Ca^{2+} influx through NMDA receptors mediates the rapidly-triggered NMDA neurotoxicity, while Na^+ influx contributes to the swelling of the neuronal cell body (*21*). Therefore, we studied the expression of NMDA receptors in this study both in sciatic nerves and spinal cord. A significant increase was detected in VCR group. Our results are consistent with another study that showed that NMDA receptor antagonist decreased VCR-induced hyperalgesia (*22*). However, it has been reported that NMDA receptor-mediated signaling pathways were not involved in VCR-induced neuropathic pain in an *in vitro* study on primary cerebellar granule neurons (*23*). This discrepancy might be due to the differences between the *in vivo* and *in vitro* studies as well as the differential vulnerability of neurons to the NMDA antagonist.

Current results showed that VCR resulted in significant reduction of CGRP expression in sciatic nerves and spinal cord. It was reported that epineurial peptidergic terminals mediate a vasodilatory response through CGRP that increases blood flow in the downstream endoneurial compartment (*24*). Thus, it may be suggested that VCR by decreasing CGRP decreases the blood flow to the nerves producing ischemia. Won *et al*. (*20*) showed that ischemia induces excitotoxicity. Therefore, VCR-induced excitotoxicity in our study may not only be due to increase in expression of NMDA receptors, but may also be due to ischemia. It appears that VCR mediated its effects at the level of both spinal cord and peripheral nerves.

In the present study, EPO1 reversed most of VCR-induced changes in all electrophysiological parameters measured, except chronaxie. It also shortened the latency of the tail flick test. EPO2 had a similar effect to EPO1, but was also able to reverse the decreased chronaxie induced by VCR. It was also more potent than EPO1 with regard to tail flick test. From these results we could suggest that the higher dose of EPO has a more pronounced effect on both C and A-alpha or A-beta fibers but this needs further investigation. Unexpectedly, EPO2 proved to be less effective on MCAP. This could not be due to a toxic effect of the high dose of EPO as the other electrophysiological recordings were completely reversed. Further studies are recommended.

In the present study, the increase in NMDA receptor expression by VCR was also significantly reduced by both doses of EPO in spinal cord and sciatic nerves. Yet, the higher dose of EPO in the EPO2 group produced a complete reversal of VCR-induced changes at the peripheral nerve level. This clearly indicates that one of the mechanisms of action of EPO as a neuroprotective agent is mediated through decreasing excitotoxicity. It also seems that the higher dose was more capable of preventing excitotoxicity as it had a more prominent effect not only on NMDA receptor expression, but also on improving the latency of the tail flick test and

chronaxie, which are both indicators of excitablity. An inhibitory effect of EPO on excitotoxicity was reported in several neurotoxic models (*25*,*26*). Furthermore, Yazihan *et al*. (*27*) showed that the neuroprotective effect of EPO was abolished *via* NMDA receptor antagonist. Other previous studies suggested that EPO could ameliorate or prevent neuronal injury by other mechanisms such as antiapoptotic, antioxidant and anti-inflammatory effects (*28*,*29*).

In the current study, the reduction in CGRP expression observed in the spinal cord and sciatic nerves with VCR administration was reversed by EPO. At the level of the spinal cord, both doses of EPO even increased CGRP expression to higher levels than normal. Similarly, Toth *et al*. (*30*) reported that EPO increased the density and intensity of CGRP expression within outgrowing axons after crush injury. Thus, it seems that another mechanism for improvement of VCR-induced neurotoxicity by EPO may be *via* restoration of blood flow in central and peripheral nervous tissue by increasing CGRP.

It can be concluded that VCR caused electrophysiological changes indicating axonal degeneration. This study was the first to demonstrate that VCR caused a state of glutamate excitotoxicity both centrally and peripherally. It could also decrease endoneural blood flow and induce vascular neurotoxicity but this needs further investigation to prove it. The higher dose of EPO was more effective in improving VCR-induced neurotoxicity. There may exist several mechanisms of action of EPO, but we were able to detect that EPO decreased NMDA receptor expression and increased CGRP expression, thus decreasing the excitotoxicity caused by VCR.

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(*Received October 20, 2010; Revised November 29, 2010; Re-revised April 13, 2011; Accepted May 19, 2011****)***