Protective Effect of Vitamin E on Nerve Conduction and Dorsal Root Ganglia Against Cisplatin-Induced Peripheral Neurotoxicity in Rats

SANDRA M. YOUNAN, M.D.* and LEILA A. RASHED, M.D.**
The Departments of Physiology* and Biochemistry**, Faculty of Medicine, Cairo University

Abstract

Background: Cisplatin is commonly used against several solid tumors. Severe peripheral neuropathy is a major clinical issue affecting 10- 40% of patients treated with cisplatin and is associated with apoptosis of sensory dorsal root ganglia. Vitamin E is one of the several substances with suggested effects on peripheral nerves but still its exact benefit is not clear. The aim of this study is to investigate the possible protective effect of α-tocopherol the active form of vitamin E on cisplatin-induced peripheral neurotoxicity in rats.

Methods: The study included four groups: Control, cisplatin, vitamin E-control and cisplatin-vitamin E groups. Twenty-four hours after the end of treatments, tail sensory nerve and sciatic motor nerve conduction velocities were conducted also the histopathology of the sciatic nerve was studied. The sciatic dorsal root ganglia (DRG) malondialdehyde (MDA), superoxide dismutase (SOD) activity, glutathione, NF-kappa B (NF-KB) and antiapoptotic Bc1-2, apoptotic Bax and caspase-3 expression as well as serum TNF-alpha were measured.

Results: Cisplatin (2mg/kg/twice weekly, in a total of eight intraperitoneal injections) significantly decreased sensory nerve conduction velocity without affecting the motor nerve conduction velocity and caused sciatic nerve axonal degeneration with loss of myelination. Cisplatin also increased dorsal root ganglia MDA level, NF-KB, Bax and caspase-3 expression as well as serum TNF-cit and decreased dorsal root ganglia superoxide dismutase activity and glutathione levels and Bcl-2 expression in the cisplatin group compared to the control group. Oral vitamin E (daily oral α-tocopherol 1000mg/kg) concomitant with the cisplatin doses, significantly improved the sensory nerve conduction velocity, preserved sciatic nerve fibers morphology, decreased dorsal root ganglia MDA level and NF-KB, Bax and caspase-3 expression and serum TNF-a and increased DRG superoxide dismutase and glutathione levels and Bcl-2 expression in the cisplatin-vitamin E group compared to the unprotected cisplatin group.

Conclusion: Vitamin E has potential neuro protective effects in cisplatin-induced sensory neuropathy linked to its anti-oxidant, anti-inflammatory and anti-apoptotic actions on dorsal root ganglia which may spark its future use in clinical settings.

Key Words: Cisplatin … Vitamin E … Peripheral neurotoxicity.

Introduction

CHEMOTHERAPY-INDUCED peripheral neurotoxicity (CIPN) is a major clinical problem because it may severely affect the quality of life of cancer patients and cause chronic discomfort [11]. Cisplatin (CIS) and the other platinum-derived drugs are among the most effective antineoplastic agents used in testicular, bladder, ovarian, cervical and endometrial solid tumors [2,3]. However, platinum drugs are severely neurotoxic which frequently requires a dose reduction or even treatment withdrawal. Neuropathies may progress for several months after cessation of cisplatin treatment and can not be treated effectively. Therefore, effective prevention strategies of CIPN would be a major advance for cancer patients [4].

The mechanism underlying cisplatinum’s anticancer activity is incompletely defined, but it is generally accepted that it is a DNA-damaging agent which forms CIS-DNA adducts that kill cells via several mechanisms, resulting in the induction of apoptosis even in normal cells [5]. Reactive oxygen species (ROS) is a recently recognized mechanism in the pathogenesis of the CIS-induced toxicity and numerous studies have shown that CIS exposure disrupts the redox balance of tissues, suggesting that biochemical and physiological disturbances result from oxidative stress [6,7]. Furthermore, platinum compounds appear to accumulate in the dorsal root ganglia (DRG) of nerves, causing its damage, apoptosis and resulting in secondary nerve fiber axonopathy. Also among the proposed mechanisms of the cisplatin toxicity is the reduction of circulating levels of nerve growth factor (NGF), a reparative neurotrophin [8].

Neuroprotective strategies might aim either at a reduced accumulation of drug-induced DNA
adducts or at improved survival or function of critical cells despite of their actual damage burden. There is increasing evidence for the protective properties of vitamin E in neuronal tissues [9]. Clinical and neuropathological features observed in human cisplatin-induced neuropathy were reported to be similar to those observed in vitamin E deficiency neuropathy with involvement of dorsal root ganglia Rol. Thus it was suggested that vitamin E may be therapeutically useful in cisplatin-induced neurotoxicity.

Vitamin E is a lipid soluble antioxidant which especially protects biological membranes from lipid peroxidation [11] and may have possible anti-inflammatory effects [12]. The antioxidant properties of vitamin E have been reported to interfere with cisplatin-induced ototoxicity [13] and nephrotoxicity [14] without interfering with cisplatin anti-tumor efficacy. Moreover, a study performed in streptozotocin-diabetic rats by Balderlou et al. [18], indicated that vitamin E seems to exert an anti-oxidant effect on the dorsal root ganglia, which is known to be the target of cisplatin neurotoxicity. Few clinical trials reported the prophylactic effect of alpha-tocopherol, the biologically active form of vitamin E against chemotherapy-induced neuropathy in patients treated with cisplatin [16,17]. Meanwhile, another study [18] reported failure of protective effect of vitamin E in chemotherapy-induced neuropathy, however, stated that a more thorough understanding of the exact role of vitamin E in cisplatin neuroprotection is needed tailored to the different mechanism of action of the neurotoxic drug.

The aim of this study is to investigate:

1) The potential prophylactic effect of vitamin E on nerve conduction in a rat model of cisplatin-induced neurotoxicity.
2) Vitamin E possible anti-oxidant, anti-inflammatory and anti-apoptotic protective effects on the dorsal root ganglion the main target of cisplatin toxicity.

Material and Methods

Animals and experimental design:

Animals were purchased from the animal care unit of Cairo Medical University and all procedures that involved animals were approved by this unit. 32 male Wistar rats, weighing 120-130g, were housed four to a cage in a constant temperature (22-24°C) and light controlled room on an alternating 12:12h light-dark cycle and had free access to food and water.

The rats were randomly divided into the following groups (n= 8/group):
Control group:
Cisplatin-group:
Receiving cisplatin in scheduled doses.
Vitamin E-control group:
Receiving daily vitamin E.
Cisplatin-vitamin E group:
Receiving cisplatin in scheduled doses and daily vitamin E.

Induction of neurotoxicity:
The cisplatin group received cisplatin (Mylan, Saint-Priest, France) in a dose of 2mg/kg intraperitoneally, twice a week, for a total of eight doses. Cisplatin was diluted in normal saline to the final concentration of 0.5mg/ml. The dilution was aimed to give excess fluid to prevent nephrotoxicity to exclude that renal failure might modulate the nerve conductivity. Physiological and structural abnormalities of peripheral nerve were previously induced with this dose and schedule of cisplatin injection [19]. The control group received only normal saline.

Vitamin E administration:
The cisplatin-vitamin E group received similar cisplatin doses concomitant with daily oral vitamin E (a-tocopherol, evitol, Kahira pharmaceutical and chemical industrial Co., Cairo, Egypt) in a dose of 1000mg/Kg [20]. The vitamin E-control group received only daily oral vitamin E.

Electrophysiology:
Sensory nerve conduction velocity (SNCV):
Twenty four hours after the end of the treatment period (i.e., on day 29) each rat underwent the determination of SNCV in the tail as described previously [21], using a method that was already used in previous experiments on neuroprotection. Briefly, the animals were anesthetized with intraperitoneal injection of ketamine (100mg/kg body weight) and placed prone on the table, with both fore and hind paws fixed on a wood board. The SNCV in the tail nerve was assessed by placing stimulating hook electrodes distally in the tail nerve, with the anode at 2mm and the cathode at 5mm from the tail tip, whereas the recording needle electrodes were placed the anode at 70mm and the cathode at 75mm from the tip of the tail. Initial recording of amplitude and latency was done for S1, then the stimulating hook electrodes were displaced at a location named S2 stimulation site with the anode at 17mm and
the cathode at 20mm from the tail tip to record amplitude and latency of S2. The latency of the potentials recorded at the two sites (S1 and S2) after nerve stimulation were determined (peak-to-peak), and SNCV was calculated by dividing the distance between stimulating electrodes by the average obtained latency difference.

All the neurophysiological determinations were performed under standard conditions in a temperature-controlled room using a physiological data acquisition system (PowerLab 4/SP; ADInstruments, Castle Hill, NSN, Australia). These distal stimulating locations were chosen because as it was shown previously that it is devoid of motor fibers, therefore its stimulation could be considered to activate sensory fibers only. Stimuli were electrical pulses of 10 mV intensity (supramaximal), 0.2 ms wide, delivered at the rate of 1/s. Three sets of subsequent recordings were always performed for reliability.

**Motor nerve conduction velocity (MNCV):**

**Operative procedure:**

The lateral aspect of the right thigh, hip, and flank of the anesthetized rat was routinely prepared, including trimming off the hair and antisepsis (20% iodine solution). The sciatic nerve was exposed through a posterolateral longitudinal straight incision going down from the greater trochanter to the lateral condyle of the femur, followed by blunt dissection between the gluteus maximus and quadriceps muscles. The entire length of the nerve was made visible and its 3 main distal branches, the common peroneal, tibial, and sural nerves, were carefully identified in the popliteal fossa. Approximately 1.5cm of the sciatic nerve was exposed above its trifurcation without injuring the epineurium.

The recordings of nerve potentials were carried out using a physiological data acquisition system (PowerLab 4/SP; ADInstruments, Castle Hill, NSN, Australia). Bipolar hook electrodes were used to stimulate the right sciatic nerve and to record the nerve potentials. For the first stimulating point, two hook electrodes were placed proximally to the sciatic notch. The recording electrode was placed distally to the sciatic nerve just before the 3 distal branches. After stimulating at the first point, the electrodes were moved to the second stimulating point, 1cm distal to the first point. Supramaximal stimuli (10 mV) were delivered to the sciatic nerve by 0.2 ms in duration, from a stimulator on a Power Lab 4/SP data acquisition system. The responses were amplified with an amplifier (BIO Amp, AD-Instruments) and stored on a computer. Scope software (ADInstruments) was used for data capture and analysis. The latencies were measured from the stimulus artifact to the onset of the negative wave deflection. Nerve conduction velocities (NCV) were calculated by dividing the distance between stimulating electrodes by the average latency difference between the onsets of the compound action potential (CAP). The amplitude of the CAP was measured from peak to peak. To determine the latency, electrical stimulation was repeated 10 times and averaged per rat [22].

**Biochemical studies:**

Fasting retro-orbital blood samples were collected and to further exclude that renal failure might modulate the nerve conductivity, serum creatinine, urea, sodium and potassium were measured in all groups. Serum urea concentration was estimated by the diacetylmonoxime assay [23]. Serum creatinine concentration was determined using Jaffe’s reaction as described [24]. Sodium and potassium ions concentrations in the serum were measured using the flame photometer [25].

Commercially available ELISA kits were used to measure serum TNF-a (Quantakine High Sensitive, R&D Systems, Minneapolis, MN, USA).

**DRG extraction and sample collection:**

The L4 and L5 dorsal root ganglia (DRG) of sciatic nerve on the left side were excised from anesthetized rats with attached sciatic nerve. Dorsal root ganglia were frozen in liquid nitrogen at —80°C and sciatic nerve was placed in paraformaldehyde for histopathological examination.

**Measurement of malondialdehyde (MDA):**

The degree of lipid peroxidation was assessed according to MDA formation, which is accepted as an index of lipid peroxidation. To measure the MDA concentration, dorsal root ganglia was placed in 1 ml phosphate buffer saline with a pH of 7.3 and then homogenized in micro tube. 20% trichloroacetique acid (TCA) was added to the dorsal root ganglia homogenate to precipitate the protein and then the mixture was centrifuged. Supernatants were collected and thiobarbituric acid (TBA) solution was added to the supernatants. After boiling for 10 minutes in water bath, the absorbance was measured. The concentration of MDA in supernatants of dorsal root ganglia homogenate was calculated using the standard curve [26].

**Measurement of glutathione (GSH):**

GSH concentration was measured from the dorsal root ganglia placed in a phosphate buffer with a pH 8.0 and then 5% trichloroacetic acid
(TCA) was added, to precipitate the protein. After centrifugation, dithiobisnitrobenzoate (DTNB) solution was added to the supernatants of the homogenate, and incubated for 1 hour. The absorbance was measured. The concentration of GSH in the dorsal root ganglia homogenate was calculated using the standard curve of bovine serum albumin solution.

Measurement of superoxide dismutase (SOD) activity:

Superoxide dismutase (SOD) activity in the dorsal root ganglia homogenate was measured through the inhibition of nitroblue tetrazolium (NBT) reduction by O2- generated by the xanthine/xanthine oxidase system. One SOD activity unit was defined as the enzyme amount causing 50% inhibition in 1ml reaction solution per milligram tissue protein and the result was expressed as U/mg protein [27].

Detection of dorsal root ganglia nuclear factor-kappa B (NF-KB), Bcl-2, and caspase-3 genes expression using real time polymerase chain reaction (RT-PCR):

RNA extraction:

Total RNA was isolated from the dorsal root ganglia tissue using trizol reagent TM (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The RNA pellet was resuspended in diacylbiocarbonate (DEPC) - treated H20. The quality and concentration of the RNA were assessed using the optical density 260/280 ratio, and only samples with ratios above 1.5 were used in the experiments.

CDNA synthesis:

Total RNA was reverse transcribed using Revert Aid Premium Reverse Transcriptase-Kit (Fermentas International Inc., Burlington, Canada). Briefly Revert Aid HMinus murine leukemic virus Reverse Transcriptase was added to deoxynucleotide triphosphates (dNTP) mix (10mM), 5x reaction buffer and random hexamer primers, the mixture was subjected to cDNA synthesis cycling condition at 37°C for 30 min and at 85°C for 5min.

Quantitative real-time-PCR:

Real-time quantitative polymerase chain reaction (PCR) was performed using ABI prism 7500 sequence detector system (Applied Biosystems, Foster City, CA) [28]. Reaction mixtures contained 10pmol/p.1 of each primer and Maxima SYBR Green qPCR Kit (Fermentas) was used: 12.5111 Maxima SYBR mix and 5.5111 nuclease-free water. An amount of Sul of template cDNA was added to each reaction mix. Quantitative PCR was performed by the following protocol of thermal cycling conditions 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of 95°C for 30 s, 60°C for 30s and 72°C for 30s. Data from real-time assays were calculated using the v1.7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative Ct method. All values were normalized to the GAPDH genes.

The primer for NF-KB was forward 5'-GCG CAT CCA GAC CAA CAA TAA C-3' and reverse 5'-GCC GAA GCT GCA TGG ACA CT- 3' [29]. The primer for Be1-2 was forward: 5' CGG-GAGAACAGGGTATGA 3'; reverse 5' CAG-GCTGGAAGGAGAAGAT 3' according to [30]; for Bax was forward: 5' TTAGTGATAAAAGTACAGTCTTT 3'; reverse 5' TTAGTGATAAAAGTACAGTCTTT 3' gene bank accession (A062449).

GADPH primer was forward: 5' TGCTGGTG-TGCTGAGTATGTCG 3'; reverse 5' TTAGTGATAAAAGTACAGTCTTT 3' gene bank accession (NM 017008).

Histopathology of the sciatic nerve:

The left sciatic nerve was carefully dissected out and stored in 4% paraformaldehyde. The nerve was washed in water, dehydrated in a series of alcohols, cleared in xylene and embedded in paraffin wax. A microtome was then used to cut the nerve into 6um sections. Sections were mounted on slides, stained with Toludene blue, analyzed by light microscopy at 400 magnification and micro imaged.

Statistics:

The results were analyzed using SPSS computer software package version 10.0 (Chicago, IL, USA). Data were presented as mean±SD and evaluated by one-way ANOVA followed by post hoc Kruskal-Wallis and Mann-Whitney U tests. Differences of p<0.05 were considered significant.

Results

Effect of cisplatin and vit E on renal function tests:

As revealed from (Table 1), there was no statistical significant difference in renal functions neither between cisplatin group and control group, nor between the cisplatin-vit E group and the vit
E-control group (p>0.05) which denote absence of cisplatin effect on renal functions in corresponding groups. Also there was no statistical significant difference in renal functions between the vit E-control group and the control group or between the cisplatin-vit E group and the cisplatin group (p>0.05) indicating absence of vitamin E effect on the renal functions. All groups showed no statistical significant difference compared to the control group.

**Effect of cisplatin and vitamin E on sensory nerve conduction velocity:**

As shown in (Fig. 1) cisplatin administration significantly decreased sensory nerve conduction velocity in the cisplatin group compared to the control group and in the cisplatin-vit E group compared to the vit E-control group (p<0.05). Vitamin E administration significantly increased sensory nerve conduction velocity in the vit E-control group compared to the control group and in the cisplatin-vit E group compared to the cisplatin group although still significantly decreased compared to the control group (p>0.05). These results highlight the ameliorative effect of vitamin E on sensory nerve conduction.

**Effect of cisplatin and vitamin E on motor nerve conduction velocity:**

As shown in (Fig. 2) cisplatin administration had no statistical significant effect on motor nerve conduction velocity in the vit E-control group compared to the control group and in the cisplatin-vit E group compared to the cisplatin group although still significantly decreased compared to the control group (p>0.05). Also vitamin E administration had no significant effect on the motor nerve conduction velocity in the vit E-control group compared to the control group and in the cisplatin-vit E group compared to the cisplatin group (p>0.05).

**Effect of cisplatin and vitamin E on dorsal root ganglia MDA, SOD and glutathione:**

As revealed from (Table 2), cisplatin administration significantly increased dorsal root ganglia MDA in cisplatin group compared to the control group and in the cisplatin-vit E group compared to the vit E-control group (p<0.05). Vitamin E administration significantly decreased dorsal root ganglia MDA in the cisplatin-vit E group compared to the cisplatin group although still significantly increased compared to the control group (p<0.05). No statistical significant difference was observed between the dorsal root ganglia MDA level of the vit E-control group and that of the control group (p>0.05).

Also (Table 2) shows that cisplatin administration significantly decreased dorsal root ganglia SOD activity and glutathione level in the cisplatin group compared to the control group and in the cisplatin-vit E group compared to the vit E-control group (p<0.05). Vitamin E administration significantly increased dorsal root ganglia SOD activity and glutathione level in the cisplatin-vit E group compared to the cisplatin group although still significantly decreased compared to the control group (p<0.05). No statistical significant difference was observed between both the dorsal root ganglia SOD activity and glutathione levels of the vit E-control group and those of the control group (p>0.05).

These results indicate the potential protective anti-oxidant effect of vitamin E against the cisplatin oxidant effect.

**Effect of cisplatin and vitamin E on serum TNF-a:**

As revealed in (Fig. 3), cisplatin administration significantly increased serum TNF-a in the cisplatin group compared to the control group and in the cisplatin-vit E group compared to the vit E-control group (p<0.05). Vitamin E administration significantly decreased serum TNF-a in the cisplatin-vit E group compared to the cisplatin group although still significantly increased compared to the control group (p>0.05). No statistical significant difference was observed between the serum TNF-a of the vit E-control group and that of the control group (p>0.05).

**Effect of cisplatin and vitamin E on dorsal root ganglia NF-κB expression:**

As showed in (Fig. 4), cisplatin administration significantly increased dorsal root ganglia NF-κB expression in the cisplatin group compared to the control group and in the cisplatin-vit E group compared to the vit E-control group (p<0.05). Vitamin E administration significantly decreased dorsal root ganglia NF-κB expression in the cisplatin-vit E group compared to the cisplatin group although still significantly increased compared to the control group (p<0.05). No statistical significant difference was observed between the dorsal root ganglia NF-κB expression of the vit E-control group and that of the control group (p>0.05).
Effect of cisplatin and vitamin E on dorsal root ganglia Bcl-2 and Bax genes expression:

Moreover, cisplatin administration significantly decreased dorsal root ganglia antiapoptotic Bcl-2 gene expression (Fig. 5) and significantly increased the DRG apoptotic Bax gene expression (Fig. 6) in the cisplatin group compared to the control group and in the cisplatin-vit E group compared to the vit E-control group (p<0.05).

Vitamin E administration significantly increased dorsal root ganglia Bcl-2 gene expression (Fig. 5) and significantly decreased DRG Bax gene expression (Fig. 6) in the cisplatin-vit E group compared to the cisplatin group although still significantly decreased and increased respectively compared to the control group (p<0.05). No statistical significant difference was observed in both the dorsal root ganglia Bcl-2 and Bax genes expression of the vit E-control group compared to those of the control group (Figs. 5, 6, p>0.05).

Effect of cisplatin and vitamin E on dorsal root ganglia caspase-3 gene expression:

As observed in (Fig. 7), cisplatin administration significantly increased dorsal root ganglia caspase-3 gene expression in the cisplatin group compared to the control group and in the cisplatin-vit E group compared to the vit E-control group (p<0.05).

Vitamin E administration significantly decreased dorsal root ganglia caspase-3 gene expression in the cisplatin-vit E group compared to the cisplatin group although still significantly increased compared to the control group (Fig. 7, p<0.05). No statistical significant difference was observed between the dorsal root ganglia caspase-3 expression of the vit E-control group and that of the control group (Fig. 7, p>0.05).

Histopathology of the sciatic nerve:

Light microscopic examination of the control group sciatic nerve specimen revealed normal nerve trunk with bundles of nerve fibers surrounded by perineum (Fig. 8 A). Meanwhile, sciatic nerve axonal degeneration, loss of myelinated nerve fibers and areas of fibrosis were observed in the cisplatin group specimen (Fig. 8 B) compared to that of the control group.

The vitamin E-control group (Fig. 8 C) showed preserved nerve morphology similar to the control group with normal myelinated nerve fibers. The cisplatinum-vitamin E group sciatic nerve (Fig. 8 D) showed mildly affected nerve fibers with slightly congested blood vessels alternating with normal fibers compared to the cisplatin group.

Table (1): Effect of cisplatin and vitamin E on renal functions tests.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Cisplatin group</th>
<th>Vit E-control group</th>
<th>Cisplatin-vit E group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum urea (mg/dl)</td>
<td>20.7±2.13</td>
<td>25.09±5.13</td>
<td>23.94±4.85</td>
<td>21.59±4.22</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.13±0.02</td>
<td>0.14±0.04</td>
<td>0.15±0.02</td>
<td>0.13±0.02</td>
</tr>
<tr>
<td>Serum Na+ (mg/dl)</td>
<td>134.7±6.07</td>
<td>135.8±7.39</td>
<td>135.9±5.96</td>
<td>136.5±5.73</td>
</tr>
<tr>
<td>Serum K+ (mg/dl)</td>
<td>3.80±0.59</td>
<td>3.92±0.54</td>
<td>4.01±0.34</td>
<td>3.46±0.51</td>
</tr>
</tbody>
</table>

*: Significant compared to the control group.
+: Significant compared to the vit E-control group.
#: Significant compared to the cisplatin group.

Table (2): Effect of cisplatin and vitamin E on dorsal root ganglia malondialdehyde (MDA) level, superoxide dismutase (SOD) activity and glutathione level.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Cisplatin group</th>
<th>Vit E-control group</th>
<th>Cisplatin-vit E group</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mg tissue protein)</td>
<td>112.6±3.2</td>
<td>198.2±29*</td>
<td>112.4±4.7</td>
<td>132.8±14.4**</td>
</tr>
<tr>
<td>SOD (p/mg tissue protein)</td>
<td>2.42±0.38</td>
<td>0.87±0.19*</td>
<td>2.51±0.54</td>
<td>1.51±0.4**</td>
</tr>
<tr>
<td>Glutathione (nmol/mg tissue protein)</td>
<td>43.01±3.48</td>
<td>25.7±5.39*</td>
<td>43.23±6.46</td>
<td>34.07±4.06**</td>
</tr>
</tbody>
</table>

*: Significant compared to the control group.
+: Significant compared to the vit E-control group.
#: Significant compared to the cisplatin group.

Fig. (1): Effect of cisplatin and vitamin E on sensory nerve conduction velocity.
Fig. (2): Effect of cisplatin and vitamin E on motor nerve conduction velocity.

Fig. (3): Effect of cisplatin and vitamin E on serum TNF-α.

Fig. (4): Effect of cisplatin and vitamin E on dorsal root ganglion NF-κB expression.

Fig. (5): Effect of cisplatin and vitamin E on dorsal root ganglia Bcl-2 gene expression.

Fig. (6): Effect of cisplatin and vitamin E on dorsal root ganglia Bax gene expression.

Fig. (7): Effect of cisplatin and vitamin E on dorsal root ganglia caspase-3 expression.
152 Protective Effect of Vitamin E on Nerve Conduction

Fig. (8): Effect of vitamin E and cisplatin on the histopathological morphology of the sciatic nerve.
Sciatic nerve specimen (x 400) stained with toludine showing bundles of normal nerve fibers surrounded by perineum in control group (A) and axonal degeneration with loss of myelination in cisplatin-group (B). Specimens show normal myelinated nerve fibers in vitamin E- Control group (C) and mildly affected nerve fibers with congested blood vessels in cisplatin-vitamin E group (D).

Discussion

Cisplatin peripheral neuropathy is a major limitation in the current treatment of cancer with platinum drugs, and its prevention is still far an unsolved issue. In this work, daily vitamin E supplementation was able to improve cisplatin-induced delay in sensory nerve conduction by an antioxidant, anti-inflammatory and anti-apoptotic effects on dorsal root ganglia.

In the present study, cisplatin (2mg/kg; i.p. for 8 doses) caused a significant delay in the sensory nerve conduction velocity without affecting the motor nerve conduction velocity in the cisplatin group compared to the control group. Since all mice were given i.p. injections of 0.5ml saline with each single dose of cisplatin and no significant differences were observed in the renal functions tests (serum urea, creatinine, Na+ and Kt) thus the observed neurotoxicity is caused by a direct effect of cisplatin and not secondary to modulation by nephrotoxicity, a common side effect of cisplatin chemotherapy. The peripheral neuropathy induced in rats by repeated administration of cisplatin is qualitatively similar to that in humans and these findings are consistent with previous ones reporting a delay in sensory nerve conduction velocity with
preserved MNCV [32]. Cisplatin-induced motor neurotoxicity was only reported on more prolonged cumulative doses (14 doses) [33].

Moreover histopathological examination of the sciatic nerve revealed significant nerve damage with axonal degeneration and loss of nerve myelination in the cisplatin group compared to the control group in agreement with a previous study [34]. Loss of nerve myelination has been suggested to be caused by cisplatin-induced apoptosis of Schwann cells [35] and may predispose to future alteration in motor nerve conduction with more accumulated cisplatin doses.

In vivo studies for screening putative neuroprotective agents reproduce more reliable results including the effects of drug metabolism, bioavailability and tissue distribution with respect to the target organs in particular. In the present work daily oral vitamin E administration in its biologically active form (α-tocopherol 1000mg/Kg) concomitant with the cisplatin scheduled doses significantly improved sensory nerve conduction velocity without affecting the motor nerve conduction and was able to preserve the sciatic nerve morphology which showed mildly affected nerve fibers and preserved myelination in the vivo model of cisplatin-vit E group compared to the unprotected cisplatin group. These findings support the clinical trials reporting the neuroprotective effect of vitamin E and the lower incidence of neurotoxicity in cisplatin treated patients supplemented with vitamin E [16,36] and contradict a clinical trial performed by Kottschade et al. [17] reporting lack of vitamin E protective effect in patients with cisplatin-induced neuropathy.

Peripheral neurotoxicity induced by cisplatin involves damage to the dorsal root ganglia which is different from other chemotherapies inducing sensory-motor distal polyneuropathy related to a reversible alteration of axonal transport [37]. The mechanism of cisplatin neuropathy is still under considerable discussion [38]. Since at least part of the cisplatin toxic effect was suggested to be attributed to the induction of oxidative stress via free radical generation and lipid peroxidation [39], we investigated oxidant effects of cisplatin on the sciatic nerve dorsal root ganglia.

The present results show that cisplatin significantly increased dorsal root ganglia MDA as an index of lipid peroxidation, significantly decreased the anti-oxidant superoxide dismutase activity and the reactive oxygen scavenger glutathione level and increased DRG NF-xB expression in the cisplatin group compared to control one. Similarly, cisplatin has been reported to induce ototoxicity via free radical generation, increased MDA and decreased both SOD and glutathione [40].

Oxidative stress effects are greater in nerve root and DRG because the blood-nerve and perineurial barriers are lower at these sites leading to neuropathy and axonal degeneration [41]. Generated ROS activates NF-xB transcription which stimulates transcription of many various genes including inducible nitric oxide synthetase responsible for the production of sustained high levels of Nitric oxide (NO) [42]. Under the simultaneous generation of NO and ROS, the cellular antioxidant capabilities are suppressed [43]. In addition, it has been demonstrated that NO may enhance cellular injury by decreasing intracellular glutathione levels one of the most important molecules in the cellular defense against chemically reactive toxic compounds and oxidative stress [44]. Decreased cellular glutathione levels and a decreased capacity for glutathione synthesis sensitize cells to certain drugs.

Interest has been focused in compounds that act as antioxidants and are capable of stimulating glutathione synthesis especially that peripheral nerve antioxidant defenses are very low compared to central nerves [45]. The current results show that vitamin E administration significantly decreased dorsal root ganglia lipid peroxidation (MDA) and the expression of the oxidative stress-responsive transcription factor NF-xB and the expression of the oxidative stress-responsive transcription factor NF-xB and glutathione level suggesting that vitamin E may decrease the oxidative environment created by cisplatin partly by enhancing other antioxidants to protect the nerve against free radical-induced damage.

These findings agree with studies reporting ability of dietary vitamin E to reduce nerve damage from oxidation products such as manoldialdehyde in diabetic rats [46]. Vitamin E was also reported to attenuate the induction of the NF-xB in liver cells even independently from its antioxidant activity [47]. Vitamin E is the major chain-breaking antioxidant present in biological membranes and fluids and an essential factor for the maintenance of the normal structure, function and integrity of the nervous system.

Clinical data have shown a clear connection between oxidative stress and the production of inflammatory mediators and that underlying inflammatory conditions may predispose patients to develop very severe toxic neuropathies. The current results show that cisplatin induced significant
increase in serum TNF-a in the cisplatin group compared to the control group. It is to be mentioned that TNF-a gene transcription is also stimulated by NF-xB [48] and that increased NF-xB is associated with chronic inflammation and neurodegeneration [49]. Vitamin E administration had an anti-inflammatory action by decreasing serum TNF-a in the cisplatin-vit E supplemented group compared to the cisplatin group providing additional protective effect of vitamin E. These results are supported by a previous study demonstrating that the administration of Vitamin E reduced the production of IL-113, IL-8 and TNF-a by leukocytes [50].

Dorsal root ganglia apoptosis is considered by some to be the main pathogenic mechanism in cisplatin-induced neuropathy [51]. In this study, cisplatin administration induced dorsal root ganglia apoptosis evident by the significant increase in DRG markers of apoptosis: Bax and caspase-3 genes expression and the significant decrease of the DRG anti-apoptotic Bc1-2 gene expression in the cisplatin group compared to the control group. Neurons have programmed cell death pathways that are particularly sensitive to DNA damage induced by many chemotherapeutic agents. Indeed it was previously shown that cisplatin induces apoptosis in dorsal root ganglion sensory neurons by covalently binding to nuclear DNA resulting in DNA damage, with subsequent Bax-mediated apoptosis via the mitochondria [52,53].

Accumulating evidence indicate that mitochondria plays a pivotal role in the apoptotic-death signaling pathway in mammalian cells. The caspase activation events could be initiated by the release of cytochrome c from the mitochondria into the cytosol and subsequently activated caspase-3 executes apoptosis by cleaving various cellular substrates vital for cell survival, ultimately resulting in apoptosis [54]. It is the expression and subcellular translocation of cell death initiators or repressors such as Bax and Bc1-2 respectively that regulate the release of cytochrome c [55]. Bc1-2 blocks cytochrome c release in response to a variety of apoptotic stimuli [56]. In contrast, the upregulation and redistribution of Bax from the cytosol to the mitochondria could promote the release of cytochrome c [57].

The membrane of mitochondria contain a high proportion of polyunsaturated fatty acyl chains [58] and may therefore be more susceptible to damage during vitamin E deficiency which was suggested to be associated with cisplatin treatment [16]. The evidence from the present study show that administration of vitamin E decreased dorsal root ganglia cisplatin-induced apoptosis indicated by the significantly decreased Bax and caspase-3 genes expression and the significantly increased Bc1-2 gene expression in the dorsal root ganglia of the cisplatin-vit E group compared to the cisplatin group. Vitamin E anti-apoptotic effect could be related to its antioxidant effect since oxidative stress is one of the main triggers of apoptosis [59] and could be also related to its ability to decrease NF-xB which previously has been shown to regulate genes and pathways responsible of cell apoptosis [R]. Furthermore, vitamin E was reported to have the ability to repair DNA damage, a property that has been suggested to be also useful in cancer prevention [61].

These findings are consistent with previous ones reporting that vitamin E pre- and co-treatment counteracted the neurotoxic effects of haloperidol on members of the Bc1-2 family such that cell survival is ensured [62]. Also similar reduction in Bax and caspase-3 with upregulation of Bc1-2 by vitamin E was reported in mouse embryonic lung cells culture antagonizing the apoptotic effect of cigarette smoking [63] and in this study an additional in vivo anti-apoptotic effect of vitamin E is suggested in the dorsal root ganglia counteracting that of cisplatin. These results may spark future beneficial effect of vitamin E in cisplatin neurotoxicity especially that an evaluation of vitamin E in a preclinical animal study found it did not interfere with the tumor inhibition or tumor growth delay of cisplatin.

Interestingly, vitamin E administration to control group had no effect on all measured parameters with the exception of the sensory nerve conduction velocity which was significantly increased in the vitamin E-control group compared to the control group.

In conclusion, in the present study vitamin E improved cisplatin-induced impaired sensory nerve conduction in rats, dorsal root ganglia MDA and glutathione levels, SOD activity as well as DRG NF-kB, Bc1-2, Bax and caspase-3 expression. Therefore, vitamin E may serve as potential protective agent in cisplatin peripheral neurotoxicity due to its potential anti-oxidant, anti-inflammatory and anti-apoptotic neuroprotective actions.

References


