

Neuroprotective Effect of Vitamin E Supplementation in Patients Treated With Cisplatin Chemotherapy

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Purpose: The aim of this study is to evaluate the neuroprotective effect of antioxidant supplementation with vitamin E in patients treated with cisplatin chemotherapy.

Methods: Between April 1999 and October 2000, forty-seven patients were randomly assigned to either group one, which received vitamin E supplementation during cisplatin chemotherapy, or to group two, which received cisplatin chemotherapy alone. Alpha-tocopherol (vitamin E; 300 mg/d) was administered orally before cisplatin chemotherapy and continued for 3 months after the suspension of treatment. For preclinical studies, nude mice carrying the human melanoma tumor were treated with cisplatin alone or in combination with vitamin E.

Results: Twenty-seven patients completed six cycles of cisplatin chemotherapy: 13 patients in group one and 14 patients in group two. The incidence of neurotoxicity was

significantly lower in group one (30.7%) than it was in group two (85.7%; $P < .01$). The severity of neurotoxicity, measured with a comprehensive neurotoxicity score based on clinical and neurophysiological parameters, was significantly lower in patients who were supplemented with vitamin E than in patients who were not supplemented with vitamin E (2 v 4.7 , $P < .01$). The results of the preclinical studies showed that when cisplatin was combined with vitamin E, no differences were observed in tumor weight inhibition, tumor growth delay, or life span as compared with treatment with cisplatin alone.

Conclusion: Supplementation of patients receiving cisplatin chemotherapy with vitamin E decreases the incidence and severity of peripheral neurotoxicity.

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CISPLATIN IS one of the most effective anticancer drugs. Renal, gastrointestinal, and neurologic toxicities have posed limits to its use; however, because hyperhydration has significantly reduced the incidence of renal toxicity, neurotoxicity remains the major dose-limiting toxicity.¹ Cisplatin-induced neurotoxicity includes peripheral sensory polyneuropathy, ototoxicity, and rarely, focal encephalopathy.² Peripheral neuropathy usually progresses for months after the end of treatment, and clinical signs and symptoms are often not reversible. The incidence of cisplatin-induced peripheral neuropathy is strongly related to the methods used to assess toxicity. Comprehensive neurologic and neurophysiologic examinations have revealed that all patients receiving cisplatin treatment with cumulative doses higher than 300 mg/m² develop signs of neurotoxicity. The mechanism of cisplatin-induced neurotoxicity is not fully understood, but the selective involvement of large myelinated sensory-fibers suggests that it may derive from dorsal-root ganglia injury.³

Neuropathologic examinations in animal models of cisplatin neuropathy have revealed pathologic changes in the dorsal-root ganglia. Similarly, pharmacologic examinations of neural tissue of human patients treated with cisplatin have shown platinum accumulation in the dorsal-root ganglia that are not protected by the blood-brain-barrier.^{4,5} In recent years, many neuroprotective agents have been investigated with the aim of reducing the negative effect of peripheral neurotoxicity on a patient's quality of life and to allow an increase in the cumulative doses of cisplatin in the treatment of chemosensitive tumors. In preclinical studies, neurotrophic factors (eg, adrenocorticotropic hormone analogs, ciliary growth factor, and nerve growth factor), oxygen free-radical scavengers, thiols, and other substances have all shown some neuroprotective effects.^{6,7} Preliminary results obtained in human studies indicate that amifostine, an organic thiophosphate compound that acts as a scavenger of oxygen

free-radicals, has some activity against cisplatin-induced peripheral neurotoxicity and renal toxicity.⁸

Some studies have focused on the role of antioxidants in the mechanism of cisplatin toxicities. Recent evidence indicates that cisplatin-induced side effects are, at least in part, the result of the formation of oxygen free-radicals.^{9,10} In animals, supplementation with antioxidants, such as vitamin E, vitamin C, and selenium, seems to protect against cisplatin-induced renal and ototoxicity.^{11,12,13} Moreover, data obtained in human studies indicates that cisplatin treatment induces a fall in plasma antioxidant levels because of oxidative stress.¹⁰ It is interesting to note that the clinical and neuropathologic features observed in cisplatin-induced neuropathy are similar to those observed in vitamin E deficiency-induced neuropathy. In humans, vitamin E deficiency syndromes (eg, lipid malabsorption, cholestatic liver disease, abetalipoproteinemia, short bowel syndrome, cystic fibrosis, and familial sporadic vitamin E deficiency) are characterized by a peripheral sensory neuropathy with ataxia, paresthesia in a stocking and glove distribution (paresthesias and numbness in feet and hands), and loss of reflexes caused either by the retrograde degeneration of the large caliber axons in peripheral nerves or by degeneration of the posterior columns of the spinal cord.¹⁴ Pathologic studies indicate that

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Table 1. Neurotoxicity Score Based on Subjective Clinical Symptoms, Clinical Signs, and Electrophysiological Findings*

Variable	Neurotoxicity Score			
	0	1	2	3
Sensory symptoms (NSS)†	None	NSS 1	NSS 2	NSS > 3
Vibratory sensation	Normal	Slightly reduced	reduced	absent
Tendon reflexes	Normal	AJ‡ reduced	AJ absent	all absent
Median sensory amplitude	Normal	10%-25% decrease	25%-50% decrease	> 50% decrease
Sural sensory amplitude	Normal	10%-25% decrease	25%-50% decrease	> 50% decrease

Abbreviations: NSS, Neurologic symptom score; AJ, angle jerks.

*Modified from Chaudry et al. (21). Neurotoxicity was based on the following scoring system: mild, less than 5; moderate, 5 to 8; severe, more than 8.

the dorsal-root ganglia neurons are the primary target of vitamin E deficiency.¹⁵

In a recent pilot study, we found a decrease in plasma levels of vitamin E in patients with severe peripheral neurotoxicity after cisplatin treatment,¹⁶ indicating that vitamin E deficiency could be involved in the mechanism of cisplatin-induced neurotoxicity. The aim of this study is to evaluate the neuroprotective effect of antioxidant supplementation with vitamin E in patients treated with cisplatin chemotherapy. Before carrying out the clinical study, we performed preclinical experiments on immunosuppressed mice, with implanted human melanoma, to assess whether the combination of cisplatin with vitamin E could modify the antitumoral efficacy of cisplatin.

METHODS

Preclinical Study

Male CD-1 nude (nu/nu) mice (6 to 8 weeks old; body weight, 22 to 24 g) were purchased from Charles River Laboratories (Calco, Italy). All procedures involving the use and care of animals have been described previously¹⁷ and were in accordance with institutional guidelines complying with national and international laws and policies. Each experimental group included six mice. A human melanoma cell line (M14), obtained and characterized as previously described,¹⁸ was used in this study. Nude mice were injected in the hind leg muscle with an M14 cell suspension of 5×10^6 cells/mouse. A tumor mass was evident in all mice on day 5 after tumor implant. Cisplatin (Cisplatin Teva, Teva Pharma, Ind. LTD, Israel) was injected intraperitoneally at 10 mg/kg (10% of the lethal dose; LD₁₀) in three consecutive daily injections (3.3 mg/kg/d). Previous experiments demonstrated that this dose scheduling was less toxic than a single injection in terms of loss of body weight and drug-related deaths. Vitamin E was administered orally at 4.3 mg/kg/d. Three cycles of vitamin E or cisplatin alone, or a combination of the two, were administered at 7-day intervals, starting from day 2 after tumor implant according to the following schedules: Schedule A: vitamin E on days 2 to 4 and 8 to 11 (first cycle), days 19 to 21 and 25 to 28 (second cycle), days 36 to 38, and days 42 to 45 (third cycle). Schedule B: cisplatin on days 5 to 7 (first cycle), days 22 to 24 (second cycle), and days 39 to 41 (third cycle). Schedule C: schedule A plus schedule B. As a control, an additional group of tumor-bearing nude mice received the same volume of diluents used to dissolve cisplatin and vitamin E for the same period of time as the combined treatment (ie, schedule C).

Clinical Study

Between April 1999 and October 2000, 47 patients with solid malignancies and a Karnofsky Performance Status between 70 to 100 were enrolled in the study. Candidates for cisplatin treatment were randomly assigned after informed consent to either the cisplatin treatment with vitamin E supplementation group (group one) or the cisplatin alone group (group two). No previous chemotherapeutic treatment or regimens including other neurotoxic drugs associated with cisplatin were permitted. Alpha-tocopherol (Ephynal, Roche, Milan, Italy; ie, vitamin E) was administered orally at 300 mg/d after

randomization but before chemotherapy in the patients in group one, and was sustained for 3 months after the cessation of cisplatin treatment. The median time between the start of vitamin E supplementation and the beginning of chemotherapy was 4 days (range, 1 to 8 days).

Neurologic and Electrophysiologic Examination

Before treatment, all patients were evaluated by two neurologists (AP and LB), with a neurologic examination consisting of a standardized history for detection of neuropathic symptoms, with an assessment of pinprick and vibratory sensations and strength and deep tendon reflexes. A follow-up neurologic examination was performed by the same neurologists, who were not blinded to treatment status, after the three cycles of cisplatin treatment and after the cessation of chemotherapy. Neuropathic signs and symptoms were scored using a questionnaire designed for the detection of sensory disturbances (eg, paresthesia, pain, and burning in feet or fingers) experienced by the patients. We adopted a modified version of the neurological symptom score (NSS) of Dick et al,¹⁹ grading the severity of symptoms as mild = 1, moderate = 2, and severe = more than 2. Before treatment, an electrophysiologic examination of all patients was carried out according to previously published methods.²⁰ Briefly, each patient was examined by surface electrodes to assess nerve conduction velocity and the amplitude of potentials of the sensory median and sural nerves. A follow-up electrophysiologic examination was performed after the end of treatment with vitamin E. The pretreatment and posttreatment values were compared. A decrease in median or sural sensory amplitude of greater than 25% (with respect to basal values) was considered abnormal. A cumulative neurotoxicity score was assigned for each patient on the basis of neuropathic signs and symptoms and on electrophysiologic changes (modified from Chaudry et al;²¹ Table 1). Severity of neurotoxicity was graded on the basis of obtained total scores as mild = 1 to 4, moderate = 5 to 8, and severe = more than 8, which corresponds to World Health Organization (WHO) neurotoxicity grades 1, 2 and 3-4, respectively. This detailed neurotoxicity scoring system allowed an objective assessment of neuropathic signs and symptoms and has been used in previous studies.^{20,22}

Analysis of Vitamin E

Plasma levels of vitamin E were measured in all patients before cisplatin chemotherapy. Alpha-tocopherol was analyzed by gas chromatography-mass spectrometry (GC-MS) on a capillary column (RTX1 Restex 30 $\mu\text{m} \times 0.20 \mu\text{m}$ [Restek, Bellefonte, PA]; internal diameter, 0.25 mm) by a selected ion monitoring technique as previously described.²³ Values obtained from the patients' serum samples were compared with normal values obtained from 50 healthy control subjects (30 males and 20 females; age range, 20 to 60 years) whose plasma levels of vitamin E were a mean value of 11 ± 2.1 mg/mL. Statistical analysis was carried out using the χ^2 test and the Student's *t* test (paired or not, as appropriate).

RESULTS

Preclinical Study

We first evaluated the antitumoral effect of cisplatin and vitamin E in a human-melanoma xenografted, immunosup-

Table 2. Antitumoral Effect of CDDP and Vitamin E in a Human Melanoma Xenografted in Immunosuppressed Mice

Schedule	Tumor Weight Inhibition (%)*	Tumor Growth Delay†	Increase in Life Span (%)‡
Vit. E	0	0	0
CDDP	39	11	28
Vit. E/CDDP/Vit. E	38	11	30

Abbreviations: CDDP, cisplatin; Vit. E, vitamin E.

*Percentage of tumor weight inhibition calculated as $1 - (\text{mean tumor weight of treated mice}/\text{mean tumor weight of controls}) \times 100$.

†Evaluated as $T - C$, where T and C are the median times (in days) for treated and control tumors to reach the same size (i.e., 1000 mg), respectively.

‡Percentage of increase in life span calculated as $[1 - \text{survival time (days) of treated mice}/\text{median survival time (in days) of control mice}] \times 100$.

pressed mouse model. As shown in Table 2, the results of the preclinical studies showed that when cisplatin was combined with vitamin E, no differences were observed in terms of tumor weight inhibition, tumor growth delay, or increase in life span of the mice compared with treatment with cisplatin alone. In particular, in the cisplatin alone group, tumor weight inhibition (39%) and tumor growth delay (11 days) were superimposable to those observed in the cisplatin plus vitamin E group (38% and 11 days, respectively). A similar behavior of the two groups of animals was confirmed by the survival data. An increase in life span of 28 and 30 days was observed in the cisplatin alone and the cisplatin plus vitamin E groups, respectively.

Clinical Study

Out of 47 patients enrolled in the study, 20 patients dropped out, 18 patients interrupted treatment after two or three cycles because of disease progression, and two patients out of group one suspended vitamin E supplementation after 1 month. Table 3 shows the characteristics of patients, cumulative dose of cisplatin administered, and clinical data. Twenty-seven patients (seven women; 20 men) were assessable for analysis because they received a cumulative dose of cisplatin higher than 300 mg/m² (13 in group one; 14 in group two). Distribution of age (median age, 57 years; range, 28 to 74 years) and pathology were similar in the two groups. Fifteen patients were affected by lung cancer, three by ovarian cancer, two by rhinopharynx cancer, two by urethral cancer, and one each by gastric, testicular, esophageal, ethmoidal, and tongue cancer. Cisplatin was administered in combination regimens on the basis of the specific tumor site as follows: lung and urethral cancer were treated with cisplatin 75 mg/m² on day 1 and gemcitabine 1,000 mg/m² on day 1 and day 8 every 3 weeks; ovarian cancer was treated with cisplatin 75 mg/m² and endoxan 600 mg/m² on day 1 every 3 weeks; head

and neck cancer was treated with cisplatin 100 mg/m² on day 1 and fluorouracil 1,000 mg/m² on day 1 to 4 every 3 to 4 weeks; ethmoidal cancer, which has a small cell histologic feature, was treated with cisplatin 25 mg/m² and etoposide 80 mg/m² on day 1 to 3 every 3 weeks; gastric cancer was treated with PELF regimen (cisplatin 40 mg/m² and epirubicin 30 mg/m² on days 1 and 5 plus fluorouracil 300 mg/m² and folinic acid 100 mg/m² on days 1 to 4 every 3 weeks). Testicular cancer was treated with PEB regimen (cisplatin 20 mg/m² and etoposide 100 mg/m² on days 1 to 5 plus bleomycin 30 mg on days 2, 9, and 16 every 3 weeks). The median cumulative cisplatin dose was similar between the two groups (median dose, 420 mg/m²; range, 330 to 550 mg/m² in group one and 225 to 600 mg/m² in group two). After cisplatin treatment, 18 out of 27 patients showed a clinical response (complete response + partial response), six patients showed stable disease, and three patients showed disease progression during chemotherapy. No significant difference between the two groups was observed in either clinical response or in systemic toxicities (no renal toxicity in either group; neutrophil counts < 1,000/mL in 38.4% of patients in group one and 50% in group two; thrombocytopenia < 50,000/mL in 7.6% in group one and 7.1% in group two). Two patients in group two developed ototoxicity.

Neurotoxicity Assessment

Before cisplatin treatment, neurologic examination was normal in all patients. The basal median value of vitamin E in the plasma was in the normal range and not significantly different between the two groups (8.06 and 7.17 mg/mL, respectively).

Group One. Neurologic examination after six cycles of cisplatin and vitamin E supplementation revealed mild signs and symptoms of peripheral neurotoxicity; four out of 13 patients (30.7%) complained of distal paresthesia and presented with a reduction in deep tendon reflexes. On electrophysiologic examination, performed after the end of chemotherapy and compared with the baseline examination, four patients had at least one abnormal finding in the median and/or sural sensory amplitude, one patient showed a decrease in both nerves (25% decrease in sural sensory amplitude and 50% decrease in median sensory amplitude), two patients had a decrease in median sensory amplitude (50% and 25%, respectively), and one patient had a decrease in sural sensory amplitude (25%). Mean neurotoxicity score in patients of group one was 2.1 ± 2.1 ; neurotoxicity was scored as mild in two patients and moderate in two patients.

Group Two. After six cycles of cisplatin, 12 out of 14 patients showed clinical signs and symptoms of neurotoxicity with the absence of deep tendon reflexes, distal paresthesia in feet and fingers, and a reduction in malleolar vibratory sensation. On electrophysiologic examination, performed after the end of chemotherapy and compared with basal values, 11 patients had a decrease in the amplitude of median and/or sural sensory nerves, three patients had a decrease of 50% in both nerves, five patients had a decrease in median sensory amplitude (three patients with 50% and two patients with 25%), and three patients had a decrease in sural sensory amplitude (two patients with 50% and one patient with 25%). Mean neurotoxicity score of group two was $4.7 (\pm 2.9)$; neurotoxicity was scored as mild in six patients, moderate in four patients, and severe in two patients.

Table 3. Characteristics of Patients

Characteristic	Group 1	Group 2
Median age years, range	58 (37 to 69)	57 (28 to 74)
Median CDDP cumulative dose/mq, range	420 (300 to 550)	420 (225 to 600)
Median basal vitamin E (mg/mL), range	8.0 (4.6 to 10.1)	7.2 (3.3 to 9.5)
Response rate, CR + PR	61.5%	72.7%

Abbreviations: CDDP, cisplatin; CR, complete response; PR, partial response.

Table 4. Clinical and Neurophysiological Data Assessed Before Treatment (Basal) and After Six Cycles of CDDP

Variables	Group 1		Group 2		P
	Basal	After CDDP	Basal	After CDDP	
Sural amplitude, mean \pm SD	15.5 \pm 6.3 μ V	13.7 \pm 5.5 μ V	14.5 \pm 8.5 μ V	13.6 \pm 9.2 μ V	
Sural SCV, mean \pm SD	51.2 \pm 4.1 m/sec	49.6 \pm 5.1 m/sec	48.5 \pm 5.6 m/sec	45 \pm 5.9 m/sec	< .01
Median amplitude, mean \pm SD	15.1 \pm 5.2 μ V	12 \pm 0.6 μ V	15 \pm 9.2 μ V	8.7 \pm 5.3 μ V	< .01
Reflexes and distal paresthesia, no. of patients	0 of 13	4 of 13	0 of 14	12 of 14	
Neurotoxicity score, mean \pm SD		2.1 \pm 2.1		4.7 \pm 2.9	< .01

Abbreviation: CDDP, cisplatin; SCV, sensory conduction velocity.

Neurotoxicity scores were significantly higher in group one than they were in group two ($P < .01$; Table 4). The incidence of neurotoxicity differed significantly between the groups (group one, 30.7%; group two, 85.7%; paired t -test and Wilcoxon sign-rank test: $P < .01$). The relative risk (RR) of developing signs or symptoms of neurotoxicity was significantly lower in group one than it was in group two (RR = 0.36; 95% confidence interval [CI] = 0.15 to 0.83; $P < .001$). The intention to treat analysis of the entire study population could not be achieved because the 20 patients who dropped out of the study (nine in group one; 11 in group two) did not stay in the study long enough to reach a cumulative neurotoxic dose of cisplatin. However, the neurologic and neurophysiologic examinations in the patients who dropped out (performed before chemotherapy) were normal. A follow-up assessment of neurotoxicity after three cycles of chemotherapy was only possible in 11 patients (five patients in group one; six patients in group two), and no subjective symptoms or neurological and electrophysiologic signs of neurotoxicity were detected.

DISCUSSION

Increasing interest in the oxidative damage induced by anti-cancer drugs and its role in the mechanism of adverse effects has recently been addressed.²⁴⁻²⁶ Cisplatin and other chemotherapeutic agents are known to generate oxygen free-radicals that induce membrane lipid peroxidation with subsequent extensive tissues damage.¹⁰ Recent studies have demonstrated a reduction in ototoxicity, renal toxicity, and hematologic toxicity in animals treated with cisplatin plus supplementation with high doses of antioxidants.^{9,11,13} In this study, we investigated the role of the antioxidant defense mechanism in peripheral neurotoxicity induced by cisplatin chemotherapy. Our *in vivo* experiment showed that administration of vitamin E does not impair the therapeutic efficacy of cisplatin.

On the basis of this finding, we proceeded to supplement cancer patients treated with cisplatin chemotherapy with vitamin E. This study showed that vitamin E supplementation reduces the incidence of cisplatin-induced neurotoxicity. Indeed, whereas 85.7% (12 of 14) of the patients treated with cisplatin chemotherapy alone developed signs or symptoms of peripheral neuropathy, only 30.7% (4 of 13) of the patients who received cisplatin chemotherapy and vitamin E experienced mild distal paresthesia and presented with abnormal changes in electrophysiologic examinations. The incidence of neurotoxicity at

cisplatin doses of more than 300 mg/m² is reported to be 85%.² Given that both groups in this study received a median cisplatin cumulative dose of 420 mg/m², the reduction of cisplatin-induced neurotoxicity that we observed was remarkable. Moreover, the severity of neurotoxicity, measured with a score assigned on the basis of clinical signs and symptoms and abnormal changes in electrophysiologic parameters, was significantly higher in patients who did not receive vitamin E supplementation. The neurotoxicity score used in this study is a detailed and comprehensive method of assessing neuropathic signs and symptoms on the basis of objective findings and is minimally, if at all, influenced by interpretation of the assessor. The response rate to cisplatin chemotherapy was not significantly different in the two groups. Therefore, our clinical study confirmed the findings of our *in vivo* study that vitamin E supplementation does not influence cisplatin antitumor activity.

Dorsal-root ganglia are thought to be the target of cisplatin-induced neurotoxicity because this neural tissue has the highest degree of platinum accumulation.⁴ Interestingly, it has been demonstrated that dorsal-root ganglia are also the most vulnerable neural structure in vitamin E deficiency neuropathies.¹⁴ This observation could explain why the peripheral neuropathy induced by cisplatin treatment is a sensory neuropathy that cannot be clinically or neurophysiologically distinguished from vitamin E deficiency neuropathy. Oxidative stress, with a reduction in plasma levels of antioxidants,²⁴ has also been demonstrated during treatment with other neurotoxic chemotherapeutic agents (eg, taxanes and vinca alkaloids); however, the mechanism of peripheral neurotoxicity induced by these drugs is quite different from that induced by cisplatin. Peripheral neurotoxicity, induced by taxanes and vinca alkaloids, is the result of an axonal sensory-motor distal polyneuropathy, which is related to a reversible alteration of axonal transport and does not involve dorsal-root ganglia. Our hypothesis, therefore, is that the peculiar ability of cisplatin to concentrate in the dorsal-root ganglia induces a depletion of vitamin E and renders the neuron bodies more susceptible to oxidative stress.

In conclusion, the results of our study indicate that vitamin E supplementation significantly protects against cisplatin-induced peripheral neurotoxicity and reduces the incidence and intensity of neuropathic signs and symptoms. Nonetheless, the efficacy of neuroprotection with vitamin E supplementation has to be assessed in larger studies.

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