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Research Article
Protective Effect of Polyethylene Glycol (PEG) 3350 on a Cisplatin-Induced Rat Model of Neuropathy

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Abstract

Background and Objective: Peripheral neuropathy is the most important side effect, leading to a decrease in the dose of cisplatin or its complete cessation in the early period. For this purpose, the study aimed to investigate the therapeutic potential of PEG in cisplatin-induced neuropathy and to measure the levels of MDA, GSH, IL-6 and TNF-α, which are key markers of lipid peroxidation and antioxidant capacity. **Materials and Methods:** Cisplatin was given to 16 rats twice a week for 4 weeks at a rate of 2.5 mg/kg/day. Cisplatin-treated rats were split into two groups. For 4 weeks, the rats in Group 1 (n = 8) received 1 mL/kg/day of 0.9% NaCl intraperitoneally, while the rats in Group 2 received 30 mg/kg/day of PEG. The control group consisted of the eight surviving rat specimens. The motor abilities of all the animals were assessed after the research. Blood samples were collected for the measurement of plasma lipid peroxidation malondialdehyde (MDA), Tumour Necrosis Factor (TNF-α), glutathione (GSH) and IL-6 levels. **Results:** Electromyography findings revealed that compound muscle action potential (CMAP) amplitude was significantly higher in the cisplatin-PEG group than in the cisplatin-saline group. Also, cisplatin-PEG treated group showed significantly lower TNF-α, MDA and IL-6 levels and higher GSH levels than the cisplatin-saline group ($p<0.01$, $p<0.001$). In addition, while the CMAP latency was decreased in the PEG-treated group, the CMAP amplitude was increased and a significant improvement was observed in the Inclined test scores. Besides, histological examinations showed an increase in axon diameter and NGF expression with PEG treatment. **Conclusion:** This study demonstrated that PEG exerts protective activity against cisplatin-induced neurotoxicity by increasing endogenous antioxidants and reducing lipid peroxidation and inflammation.

Key words: Cisplatin, neuropathy, polyethylene glycol, PEG, electromyography, oxidative damage, inflammation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antineoplastic drugs commonly cause Chemotherapy-Induced Peripheral Neuropathy (CIPN). The pathogenesis of CIPN is influenced by a variety of factors, including mitochondrial dysfunction, oxidative stress, Damage-Associated Molecular Patterns (DAMPs) and the expression of neuroimmune ion channels[1,2]. Neurotoxicity, ototoxicity and nephrotoxicity are associated with the use of cisplatin in the treatment of lung, genitourinary and gastrointestinal malignancies[3,4]. These include peripheral neuropathy, which can cause a reduction in the cisplatin dose or perhaps a total stop in the early stages of treatment[3,5]. The pathophysiology of cisplatin-induced neurotoxicity has been linked to oxidative stress and inflammation[3].

Motor and autonomic involvement may be present in varying degrees and for varying lengths of time in peripheral neuropathy[6]. Chronic pain and irreparable nerve damage are among the many symptoms that can occur as a result of heat stimuli. There is no known preventative cure or effective treatment for peripheral neuropathy produced by antineoplastic drugs[6,7].

Fusogens, such as polyethylene glycol (PEG), are synthetic biocompatible polymers that cause cell fusion by increasing the size of the cells and altering their membranes[8,9]. The PEG has been found to repair cell membranes and reduce oxidative stress when administered directly to the damage site[9]. This can be done by mending the plasma membranes, particularly those of axons[10,11] or by directly interfacing with mitochondria[12-14].

In cisplatin toxicity, proinflammatory cytokines such as Tumour Necrosis Factor-alpha (TNF-α), interleukin (IL)-1 and IL-6 are increased[3,12]. Hypoxia, inflammation and the buildup of reactive oxygen species (ROS) in tissues all cause apoptosis, which is a critical mechanism in cisplatin neurotoxicity[3]. Glutathione (GSH) is a tripeptide that is involved in the pathophysiology of several disorders, including redox balance, oxidative stress reduction, metabolic detoxification and immune system regulation. Because of these qualities, GSH is also thought to be a therapy target[13]. Malondialdehyde (MDA) is a byproduct of lipid peroxidation induced by the generation of reactive oxygen species (ROS). In some clinical circumstances, the oxidant-antioxidant balance might be disturbed in favour of the oxidants, resulting in oxidative stress[14,15]. The TNF-α is well-known for its function in tumour genesis and progression[16]. The inflammatory cytokine IL-6 has a wide range of biological effects[17]. The PEG is anticipated to provide positive effects in the treatment of neuropathy, according to new evidence on the aetiology of cisplatin-induced neuropathy and findings from animal research[3,5,6].

As a result, the current investigation used electromyography (EMG) recordings to assess the therapeutic potential of PEG in cisplatin-induced neurotoxicity and to measure the levels of MDA, GSH, IL-6 and TNF-α, which are key markers of lipid peroxidation and antioxidant capacity.

MATERIALS AND METHODS

Study area: The study was carried out at the Experimental Animals Application and Research Center, Demiroğlu Bilim University, Istanbul, Turkey from July, 2021 to January, 2022.

Animals: The study used 24 mature female Wistar rats weighing 200-210 g. The animals were kept in cages and subjected to conventional care, including 12 hrs light/dark cycles at ambient temperature (22±2°C). Throughout the trial, they were provided with a regular pellet meal and free access to tap water. Demiroğlu Bilim University's Institutional Animal Care and Ethical Committee gave their approval to the study's protocol (Ethical Number: 21210410). Unless otherwise stated, all compounds were purchased from Sigma-Aldrich Inc.

Experimental procedure: A total of 24 rats were used for this study. A normal control group of eight rats was included in the investigation. This group did not get any medication.

To produce neuropathy, 16 rats were administered cisplatin at a rate of 2.5 mg/kg/day twice a week for 4 weeks (a total dose of 20 mg kg^{-1}). Cisplatin-treated rats were separated into two groups. For 4 weeks, Group 1 rats (n = 8) were administered 1 mL/kg/day 0.9% NaCl (saline) i.p. and Group 2 rats (n = 8) were given 30 mg/kg/day PEG i.p. During the study, two of the rats given Cisplatin and Saline perished. In rats given cisplatin plus PEG, there was no death.

All animals were assessed for motor function and EMG after the study. The rats were then sacrificed using a cervical dislocation operation while under a high dose of anaesthetic. Biochemistry was performed on the blood.

Measurement of lipid peroxidation (MDA): Malondialdehyde (MDA) levels as thiobarbituric acid reactive compounds were used to measure lipid peroxidation in plasma samples (TBARS). Plasma samples were combined with trichloroacetic acid (TCA) and the TBARS reagent (TBARs) and incubated for 60 min at 100°C. Centrifuged for 20 min at 3000 rpm, the supernatant was examined at 535 nm for its absorbance. Tetraethoxypropane was used to calibrate the MDA concentrations, which were given as nM.

Measurement of tissue glutathione (GSH) levels: Ellman's spectrophotometric approach was used to assess GSH levels in plasma samples. Coloured anion is formed when thiols react with 5-dithiobis-(2-nitrobenzoic acid) and the greatest peak at 412 nm was observed. Based on a standard calibration curve, the GSH concentrations were estimated and denoted by μM.

Measurement of plasma TNF-α, IL-6 levels: Blood samples were tested for TNF-α and IL-6 using ELISA test kits, which may be obtained over the counter (Biosciences).

Electrophysiological recordings: Electromyography recordings were undertaken ten days after cisplatin treatment. Neuromodulation of the right sciatic nerve was performed using a bipolar subcutaneous needle stimulation electrode (BIOPAC Systems Inc., Santa Barbara, CA) with a sampling rate of 40 kHz and an intensity of 10 V. Two or three interosseous muscles were stimulated using unipolar, platinum electrodes that recorded CMAP (compound muscle action potentials). Biopac Student Lab Pro version 3.6.7 software (BIOPAC Systems, Inc.) was used to evaluate the data, with CMAP amplitude as the parameter. Rats' rectal temperatures were kept between 36 and 37°C during EMG recordings using an HP Viridia 24-C rectal probe (Hewlett-Packard Company, Palo Alto, CA) and a heating pad. Biochemical tests required the euthanasia of animals whose EMGs had been recorded and whose blood had been drawn via heart puncture. They were stored at -20°C until the assay and centrifuged at 3000 rpm for 10 min at room temperature.

Assessment of motor function: According to Rivlin and Tator's procedure, the rats' motor abilities were examined using an inclined-plate test. The rat was put on an inclined plate with its long axis perpendicular to the long axis of the inclined plate. Initially, the sloped plate had a 10-degree angle. It was necessary to increase the angle of the inclination gradually to measure the rat's ability to hold its position for 5 sec. Each rat's inclined plate angle was measured three times to arrive at a mean value for this experiment.

Statistical analysis: Statistical evaluation was performed using SPSS version 15.0 for Windows. All data were evaluated by one-way analysis of variance. *Post hoc* Tukey's HSD test was used for *post hoc* multiple comparisons. Also, the groups of nonparametric variables were compared using the Mann-Whitney's U test. In addition, the Shapiro-Wilk test was used for parametric-nonparametric differentiation. Results are presented as Mean+SEM. A $p<0.05$ was accepted as statistically significant.

RESULTS

Electrophysiology analysis: The alterations in EMG recordings in all groups were shown in Fig. 1. In this study, CMAP amplitude was found to be significantly lower in the cisplatin-saline group compared to the control group ($p<0.05$). CMAP amplitude was found to be significantly higher in the cisplatin-PEG group compared to the cisplatin-saline group ($p<0.05$) (Table 1).

The CMAP latency was statistically significantly higher in the cisplatin-saline group compared to the control group ($p<0.05$). However, CMAP latency was found to be significantly lower in the cisplatin-PEG group compared to the cisplatin-saline group ($p<0.05$) (Table 1).

While the inclined plane score was significantly lower in the cisplatin-saline group compared to the control group ($p<0.01$), it was significantly higher in the cisplatin-PEG group compared to the control group cisplatin-saline group ($p<0.05$) (Table 1).

Plasma malondialdehyde, glutathione, tumor necrosis factor-α and IL-6 levels: The MDA, TNF-α and IL-6 levels were significantly higher in the cisplatin-saline group compared to the control group ($p<0.001$). In the cisplatin-PEG group, these values were significantly lower than those in the cisplatin-saline group (IL-6 and TNF-α: $p<0.05$, MDA: $p<0.001$) (Table 2).

The GSH was significantly lower in the cisplatin-saline group compared to the control group ($p<0.01$). In the cisplatin-PEG group, GSH level was significantly higher than those in the cisplatin-saline group ($p<0.05$) (Table 2).

Table 1: Effects of PEG on CMAP latency, CMAP amplitude and inclined plane score in all groups

Parameters	Control group	Cisplatin+saline group	Cisplatin+30 mg kg^{-1} PEG group
CMAP latency (ms)	2.13±0.02	2.68±0.06 (p = 0.04)*	2.11±0.06 (p = 0.03)$^{\#}$
CMAP amplitude (mV)	13.10±0.10	4.20±0.30 (p = 0.01)*	7.90±0.80 (p = 0.02)$^{\#}$
Inclined plane score (°)	91.80±5.30	61.50±9.40 (p = 0.006)**	74.40±6.50 (p = 0.03)$^{\#}$

Results were presented as Mean±SEM, *p<0.05, **p<0.01: Different from control group and $^{\#}$p<0.05: Different from cisplatin+saline group

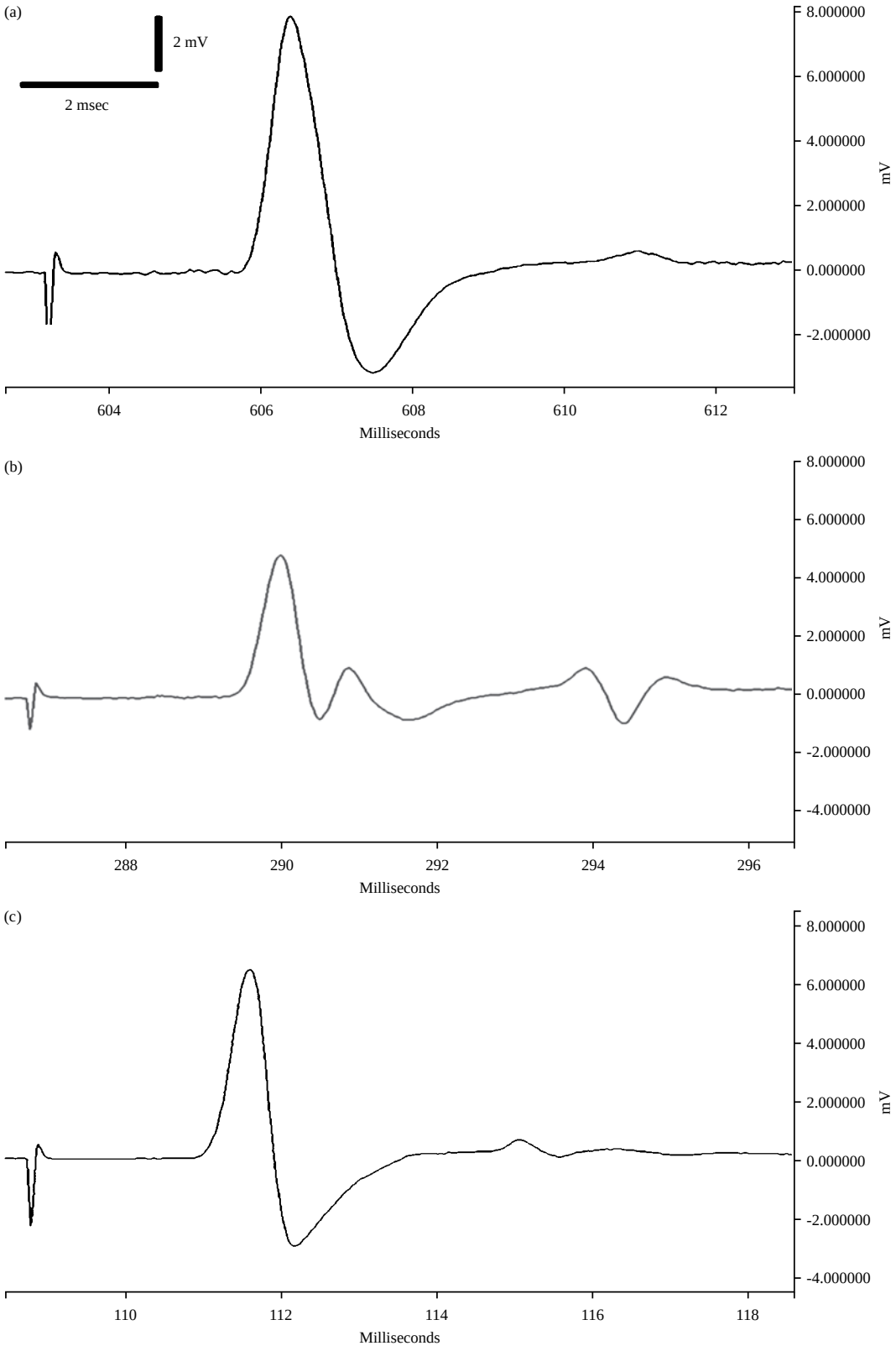

Fig. 1: Representative images of EEG recordings from each group, (a) Control group, (b) Cisplatin+saline group and (c) Cisplatin+PEG group
X-axis: Time (millisecond) and Y-axis: mV (microvolts)

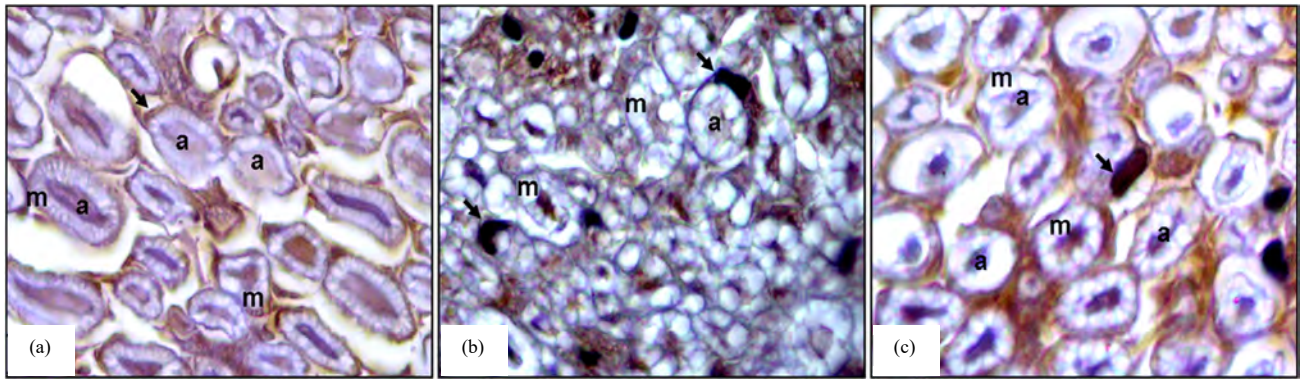

Fig. 2(a-c): NGF immunohistochemistry, (a) Normal group, (b) Cisplatin+saline group and (c) Cisplatin+PEG group

(a) Axon, arrow: Schwann cell, (b) Decreased axon diameter and NGF expression, degenerated myelin sheath and (c) Increased axon diameter and NGF expression, improved myelin sheath, (m): Myelin sheath and a-c: ×100 magnification

Table 2: Effects of PEG on plasma TNF-α, MDA, IL-6 and GSH levels in all groups

Parameters	Control group	Cisplatin+saline group	Cisplatin+30 mg kg^{-1} PEG group
MDA (nM)	51.1±7.4	148.2±14.5 (p = 0.0001)**	103.80±6.60 (p = 0.0008)##
TNF-α (pg mL^{-1})	21.5±3.5	78.9±7.30 (p = 0.0002)**	62.50±4.90 (p = 0.03)#
IL-6 (pg mL^{-1})	9.8±1.3	640.3±21.2 (p = 0.0001)**	313.70±36.5 (p = 0.02)#
GSH (μM)	13.4±2.6	5.7±0.90 (p = 0.008)*	9.02±1.10 (p = 0.02)#

Results were presented as Mean±SEM, *p<0.01, ** p<0.001: Different from control group and # p<0.05, ## p<0.001: Different from cisplatin+saline group

Table 3: Effects of PEG on plasma NGF expression and axon diameter in all groups

Parameters	Normal control	Cisplatin+saline group	Cisplatin+30 mg kg^{-1} PEG group
NGF expression (%)	93.70±5.90	34.4±7.10**	69.40±7.20##
Axon diameter (μm)	3.71±0.45	2.3±0.31*	2.98±0.54#

Results were presented as Mean±SEM, statistical analyses were performed by one-way ANOVA test, *p<0.05, **p<0.01: Different from control group and #p<0.05, ##p<0.001: Different from cisplatin and saline group

Immunohistochemical examination of sciatic nerve tissue samples: While NGF expression was significantly lower in the cisplatin-saline group compared to the control group (p<0.01), it was significantly higher in the cisplatin-PEG group compared to the cisplatin-saline group (p<0.001). While axon diameter was significantly lower in the cisplatin-saline group compared to the control group (p<0.05), it was significantly higher in the cisplatin-PEG group compared to the cisplatin-saline group (p<0.05) (Table 3, Fig. 2).

DISCUSSION

In this study, the beneficial effects of PEG on cisplatin-induced neurotoxicity using both EMG recordings and antioxidant/oxidant levels in rats were demonstrated. Uchino *et al.*[4] examined the benefits of PEG in cisplatin-induced neurotoxicity. However, to our knowledge, the current study is the first to analyze EMG and antioxidant/oxidant levels to evaluate the results of PEG application in cisplatin-induced neurotoxicity.

Antioxidant medications have been demonstrated to protect against cisplatin-induced neurotoxicity in previous clinical and experimental studies[18,19]. The PEG's neuroprotective effect on diverse neurological lesions has been demonstrated in a rising number of animal studies in recent years[3,5,10,11]. This neuroprotective effect was considered to be associated with the role of PEG in the inhibition of lipid peroxidation, elevation in antioxidant capacity and anti-inflammatory activity[3,10,11]. Luo and Shi[20] and Luo *et al.*[21] reported that the administration of PEG after acute traumatic spinal cord injury was effective in repairing damaged membranes and inhibiting apoptosis and oxidative stress. Luo *et al.*[11] suggested that in the traumatic spinal cord injury model, calcium influx into the cell caused by mechanical damage to the cell membrane resulted in an excessive increase in ROS production. The authors also reported that PEG improved the swelling of mitochondria, which is the ROS production site and repaired the damaged cell membrane by suppressing oxidative stress[10]. Similarly, Baptiste *et al.*[10] reported that PEG spread to the damaged spinal cord within

24 hrs after the application and had a therapeutic effect. In the same study, the authors presented evidence that the lipophilic properties of PEG also contributed to the healing process[9]. In this study, we hypothesized that cisplatin-induced neuronal damage in rats could be healed with PEG, which has been shown to suppress oxidative stress. To our surprise, we discovered that the cisplatin-PEG group had much higher antioxidant levels and significantly lower levels of lipid peroxidation indicators.

Akman *et al.*[3] analyzed the benefits of oxytocin in cisplatin-induced neurotoxicity with EMG and antioxidant/oxidant levels in an experimental rat model and reported that oxytocin increased the antioxidant activity, decreased lipid peroxidation and showed an anti-inflammatory effect. In this study, untreated rats given cisplatin-saline had significantly higher MDA, TNF-α and IL-6 levels, while untreated rats given cisplatin-saline had significantly lower GSH levels. In the cisplatin-PEG group, however, MDA, TNF-α and IL-6 levels were much lower, while GSH levels were significantly greater compared to the cisplatin-saline group. The findings of the current study regarding the oxidant/antioxidant balance indicated that the cisplatin-PEG group showed improvement in favor of the antioxidant and anti-inflammatory balance compared to the cisplatin-saline group. Akman *et al.*[3] obtained EMG recordings in rats administered with cisplatin-saline and cisplatin-oxytocin and found that the CMAP amplitude was significantly higher in the group that received cisplatin 160 g kg^{-1} oxytocin compared to the group that received cisplatin-saline. However, the authors found no significant difference between the two groups concerning CMAP latency[2]. In this study, CMAP amplitude was significantly higher in the cisplatin-PEG group compared to the cisplatin-saline group and unlike in the study by Akman *et al.*[3], the CMAP latency value was found to be significantly lower in the cisplatin-PEG group compared to the cisplatin-saline group. This study revealed that cisplatin-saline considerably reduced inclined plane scores when compared to controls, but cisplatin-PEG dramatically increased inclined plane scores when compared to the former.

CONCLUSION

By boosting endogenous antioxidants and lowering inflammation and lipid peroxidation, PEG can protect against cisplatin-induced neurotoxicity, according to an EMG study. For clinical and experimental studies, PEG therapy of cisplatin-induced neurotoxicity could be a model.

SIGNIFICANCE STATEMENT

Oxidative stress is one of the most critical processes involved in the development of Cisplatin-Induced Peripheral Neuropathy (CIPN), which is a typical dose-limiting adverse effect in chemotherapy patients. Fusogens, such as polyethylene glycol (PEG), are biocompatible synthetic polymers that induce cell fusion by enlarging the cells and changing their membranes. When delivered directly to the injury location, PEG has been demonstrated to mend cell membranes and decrease oxidative stress. This can be accomplished via repairing the plasma membranes, namely those of axons, or by interacting directly with mitochondria. To counteract the neurotoxic effects of cisplatin, this study found that PEG increased levels of endogenous antioxidants and decreased levels of lipid peroxidation and inflammation. In the future, PEG may be used in conjunction with other standard medications as an adjuvant treatment. Nevertheless, more investigation is needed to discover if it will be effective as a potent anti-neuropathic therapy in the clinic.

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