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Review paper

Cisplatin-associated neurotoxicity: can it be prevented?

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Neurotoxicity remains the major dose-limiting toxicity of cisplatin. Peripheral sensory neuropathy, the primary type of cisplatin neurotoxicity, has been reported in 30–100% of patients with clinical symptoms typically developing after cumulative cisplatin doses of ≥300 mg/m^2. Several clinical studies have established an important dose–response, dose intensity–response and cumulative total dose–response relationship for cisplatin in the treatment of head and neck, testicular, melanoma, and ovarian cancers. In patients with these tumor types, the occurrence of moderate or severe neuropathy presents an important therapeutic dilemma. Several types of agents—including micleophilic sulfur thiols, neurotrophic factors, phosphonic acid antibiotics and free oxygen radical scavengers—have been investigated for amelioration of cisplatin-related neurotoxicity. Of these, amifostine is likely to be the first neuroprotective agent widely used to enhance the clinical effectiveness of cisplatin. Recently reported results from a multicenter phase III trial of women with advance ovarian cancer receiving combination chemotheraphy with cisplatin plus cyclophosphamide showed that amifostine pretreatment was associated with moderate but significant reductions in cisplatin-associated peripheral neuropathy, tinnitus and nephrotoxicity, while achieving equivalent pathological response rates and median survival. Preclinical data suggest that several additional agents, especially the neurotropic factors nerve growth factor, insulin-like growth factor-I and neurotrophin-3, merit further investigation. Nerve growth factor is the only agent reported to prevent, rather than partially protect, cisplatin-induced neuropathy in an experimental model.

Key words: Cisplatin, neurotoxocity, prevention.

Introduction

Cisplatin is one of the most effective chemotherapeutic agents available for several solid tumors.[1,2] However, renal, gastrointestinal and especially neurologic toxicities have limited its use. Initially, nephrotoxicity was the major dose-limiting toxicity, but careful saline hydration with concurrent mannitol or lasix diuresis for standard cisplatin doses[3–7] and hypertonic saline with chloresis for 200 mg/m^2 cisplatin doses[8] were found to abrogate this problem. Also, the severe nausea and vomiting associated with cisplatin use largely have been controlled with high-dose metoclopramide, given alone or in combination with dexamethasone and other agents,[9,10] and especially with serotonin antagonists.[11–13] Clearly, neurotoxicity remains the major dose-limiting toxicity of cisplatin.

Cisplatin neurotoxicity

The spectrum of cisplatin-induced neurotoxicity includes peripheral sensory neuropathy,[14–20] ototoxicity,[21–24] autonomic neuropathy manifested by orthostatic hypotension and gastric paresis,[25,26] Lhermittes symptom,[27] and, rarely, focal encephalopathy,[28,29] often accompanied by cortical blindness.[28–32] Of these, peripheral sensory neuropathy is by far the most common dose-limiting toxicity.

The incidence of cisplatin-associated neuropathy is unknown. A review of published literature found neuropathy reported in 30–100% of patients, with an average of 57%.[33] The variance in development of neuropathy in these studies may reflect differences in patient populations, individual and cumulative cisplatin dose, prior or concurrent therapy, measurement techniques, or other factors. A review of published literature found an overall incidence of 9% (range 0–33%) for cisplatin-induced tinnitus, 6%

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for clinical hearing loss and 24% (range 0–90%) for audiogram abnormalities.[34]

Cisplatin-induced peripheral sensory neuropathy

Prospective studies have confirmed that peripheral sensory neuropathy is the primary type of cisplatin neurotoxicity. Neurotoxicity is dose-dependent, with symptoms typically developing after cumulative doses of 300 mg/m^2 or greater.[19,35–38] A review of published literature[35] found that neurotoxicity occurred at doses of 300 mg/m^2 or greater in 85% of patients, while only 15% became neurotoxic at doses under 300 mg/m^2. Initial symptoms are usually numbness and tingling in the distal fingers and toes and extending proximally with increasing cumulative dose. If cisplatin therapy is continued, the sense of joint position becomes impaired, resulting in more severe neurologic symptoms, including ataxia, gait disturbances, loss of manual dexterity and becoming wheelchair bound. The sensations of pain and temperature remain relatively well preserved. Symptoms may begin and often progress up to 4 months or more after cisplatin has been discontinued.[19,20,37,39–41] In 30–50% of patients, cisplatin neuropathy is irreversible, even years after cessation of treatment.

The features of cisplatin neuropathy are consistent with damage predominantly to large myelinated sensory fibers. Electrophysiologic studies have revealed an abrupt loss of sural-nerve potentials, a marked decrease in sensory neuronal conduction velocities and the preservation of motor-nerve function.[19,20,37,38,42–44] Pathological examinations have generally revealed degeneration of large axons with a secondary loss of large myelinated fibers,[20,45] though a few reports described segmental demyelination.[46,47] Morphometric analysis has confirmed a disproportionate loss of large myelinated fibers.[48]

Cisplatin-induced neuropathy is thought to be secondary to dorsal root neuronal involvement.[45,49] Pharmacologic analyses of neural tissues obtained at autopsy of cisplatin-treated patients revealed a linear relationship between platinum levels and cumulative cisplatin dose for peripheral nerve, dorsal root and dorsal root ganglia, but showed no platinum accumulation in central neural tissues protected by the blood–brain barrier (spinal cord and frontal lobe).[50] Pathologic changes in the neural tissues were found to correlate with levels of platinum within the peripheral neural tissues and observed histopathologic toxicity matched an index of exposure to platinum, defined by the product of cumulative platinum dose and the logarithm of time. All individuals who had survived for more than 100 days from first cisplatin exposure showed pathologic evidence of neurotoxicity. Platinium appeared to be retained indefinitely within nerve tissues in an actively neurotoxic form. Results were consistent with the accumulation of platinum within peripheral neural tissues producing histologic toxicity, followed by clinical toxicity as levels of platinum increase.

Little is known about the pathophysiology of cisplatin neuropathy. The fact that cisplatin neuropathy progresses for weeks after the last dose is administered suggests that axoplasmic transport is involved in the mechanism of toxicity.[51] In an experimental model of vitamin E deficiency, which presents symptoms similar to those of cisplatin neuropathy, fast antegrade and retrograde axonal transport was reduced.[52] In an experimental model of pyridoxine intoxication, which presents symptoms of sensory ataxia with relatively well preserved motor function, increasing doses of pyridoxine were associated with necrosis of dorsal root ganglion sensory neurons, accompanied by a progression from atrophy of proximal axons to atrophy of central and peripheral distal axons to axonal degeneration.[53] In the ferret model, cisplatin-induced nerotoxicity was associated with pathologic changes within the dorsal root ganglia.[54–56] At concentrations (1–10 μg/ml) similar to those associated with peripheral neuropathy in humans, cisplatin caused both neuronal and Schwann cell injury in the rat embryo dorsal root ganglion model.[57]

Cisplatin-induced ototoxicity

Cisplatin-induced ototoxicity becomes clinically important in most patients when cumulative doses exceed 400 mg/m^2 in adults[58–60] or 200 mg/m^2 in children.[61] However, audiometric testing of adults with advanced disease but normal hearing showed that all patients experienced hearing losses in the ultra-high frequency range (9000–20 000 Hz) after just one or two courses of high-dose cisplatin (150–225 mg/m^2)[23] and in a small percentage of patients at standard doses of cisplatin. With repeated doses of cisplatin[21,22,24] or after only one or two courses of high-dose cisplatin (>120 mg/m^2),[23,62] hearing loss was detected in the high frequency range (4000–

8000 Hz), as well. Cisplatin ototoxicity may include tinnitus, otalgia and, in rare cases, vestibular alterations,[63] in addition to hearing loss. Although tinnitus is reversible, cisplatin-induced hearing loss is progressive and irreversible.

Work with experimental animals has shown that cisplatin-induced ototoxicity is due to histopathologic damage at the peripheral receptor level,[64,65] characterized by widespread outer hair cell loss in the organ of Corti.

Cisplatin dose escalation

Several clinical studies have established an important dose–response, dose intensity–response and cumulative total dose–response relationship for cisplatin in the treatment of head and neck,[66] testicular,[67] melanoma,[68] and ovarian cancers.[16,69–73] In patients with these tumor types, the occurrence of moderate or severe neuropathy presents an important therapeutic dilemma. A cytoprotective agent that can delay the onset of neurotoxicity until a higher cumulative dose of cisplatin has been administered may, therefore, dramatically improve the prognosis for selected patients.

Cytoprotective agents

Ideally, a cytoprotective agent would prevent cisplatin-related side effects without affecting the platinum–DNA adducts responsible for antitumor activity, and without introducing new toxicities. Several types of agents that have been investigated for amelioration of cisplatin-related neurotoxicity, discussed below.

Nucleophilic sulfur thiols

Sulfhydryl-containing compounds—including amifostine, diethylthiocarbamate, glutathione and sodium thiosulfate—have been shown to reduce the toxicity of radiation therapy, and alkylating agent and platinum chemotherapy.[74–79] There are two possible mechanisms by which thiol may modulate cisplatin toxicity. Since sulfur is a strong nucleophile which has a high affinity for platinum, it may interact with the electrophilic active site of cisplatin, inactivating the active platinum species in circulation. Alternatively, the thiol may displace or extract platinum from the target site or from cisplatin–protein complexes. *In vitro*, various thiol-containing cytoprotective agents were shown to interfere with the formation of cisplatin–DNA adducts during co-incubations, as well as reverse a small part of the formed cisplatin–DNA adducts during post-incubations.[74,80] Diethylthiocarbamate and sodium thiosulfate, but not the active metabolite of amifostine, were able to rapidly reverse the platinum–methionine-like bonds in model compounds.[81]

Neurotrophic factors

The neuropeptide Org2766, an ACTH analog without corticotropic or melanotropic activity, has restored nerve function over a period of several months in experimental models of sciatic nerve crush lesion[82–86] and prevented cisplatin-induced peripheral neuropathy in a rat model.[87–89] Histological and functional studies suggest that Org2766 increases the number of newly formed nerve sprouts at the site of the lesion,[83,85] perhaps by mimicking or amplifying an endogenous signal that operates early in the regenerative response of the damaged neuron.[90,91] Preliminary clinical studies of Org2766 in women receiving cisplatin/cyclophosphamide therapy for advanced ovarian cancer showed a decreased incidence and severity of cisplatin-induced neuropathy with no apparent detrimental effect on antitumor activity.[92] However, the results of a recent phase III trial of Org2766 has not confirmed its neuroprotective effects.[93]

Nerve growth factor, a polypeptide involved in the development of dorsal root ganglion sensory neurons and in the maintenance of normal ganglion function in adults, prevented cisplatin-induced neuropathy in a mouse model.[94] The neurotrophic factors insulin-like growth factor-I (IGF-I) and neurotrophin-3 (NT-3) also have been shown to prevent drug-induced peripheral neuropathies in mouse and rat models.[95,96]

Phosphonic acid antibiotics

Fosfomycin, a phosphonic acid antibiotic, protects against aminoglycoside-induced ototoxicity with a concomitant reduction in histologic damage in the inner ear in both animals and humans.[97,98] The mode of action for this effect is unknown. In animal models, co-administration of fosfomycin significantly reduced cisplatin-induced ototoxicity and histologic damage in the inner ear.[99–102]

Free oxygen radical scavengers

Generation of free oxygen radicals and lipid peroxides have been implicated as the cause of cisplatin-induced renal tubular injury[103] and destruction of brain tissue in central nervous system injury. Non-glucocorticoid 21-amino steroid derivatives which inhibit lipid peroxidation and scavenge oxygen free radicals have been found effective in treating experimental models of spinal cord injury and spinal trauma,[104,105] experimental concussive head injury,[106] cerebral ischemia,[107] and ischemia-induced cochlear injury,[108] and in reducing cisplatin-induced ototoxicity.[102]

Data summarized in Table 1 for individual agents suggest that several agents—including amifostine and experimental neurotrophic factors—may be effective in reducing the incidence and severity of

Table 1. Experimental chemoprotectants for cisplatin-induced neurotoxicity

Agent	Chemoprotectant activity	Limitation(s)
Nucleophilic sulfur thiols		
amifostine	significantly reduced cisplatin-induced neurotoxicity, tinnitus and nephrotoxicity in ovarian cancer patients receiving cisplatin + cyclophoshamide chemotherapy, without affecting pathologic CRs or median survival[132,133] preliminary studies suggest amifostine enhancement of cisplatin activity in patients with non-small cell lung cancer receiving cisplatin + vinblastine chemotherapy[135,139,140]	amifostine-induced hypotension may limit use in patients with head and neck, esophageal, and non-small cell lung cancers, or with prior neck irradiation or hypercalcemia[143]
diethylthiocarbamate	reduced cisplatin nephrotoxicity in animal models[144–146]	human trials stopped due to severe but reversible autonomic hyperactivity[148–152]
glutathione	preliminary comparative studies showed reduction in neurotoxicity and potential enhancement of cisplatin activity in patients with gastric cancer,[167] but not in ovarian cancer patients[159]	
sodium thiosulfate	reduced cisplatin nephrotoxicity in animal models[177] and in preliminary clinical studies[182,183]	preliminary clinical trials failed to show neuroprotective effect[184,185] locoregional therapy only, because it has been shown to neutralize cisplatin-induced cytotoxicity[181]
Neurotrophic factors		
Org2766	stimulates formation of nerve sprouts at site of lesion[84,85,169] prevented cisplatin neuropathy in animal models[87–89,171]	comparative studies failed to show reduced cisplatin neuropathy in ovarian cancer patients receiving cisplatin + cyclophosphamide chemotherapy[93]
NGF	prevented cisplatin neuropathy in animal models[94]	no human trials
IGF-1	prevented cisplatin neuropathy in animal models[95,96]	no human trials
neurotrophin-3	prevented cisplatin neuropathy in animal models[95]	no human trials
Phosphonic acid antibiotics		
fosfomycin	reduced cisplatin ototoxicity in animal models[99–102]	no human trials mechanism of action unknown
Free oxygen radical scavangers		
Lazaroid U74006F	reduced cisplatin ototoxicity in animal models[102]	no human trials

cisplatin-induced neurotoxicity without adversely affecting antitumor efficacy, reflected by overall response rates. However, prospective, randomized, comparative clinical studies will be required to clarify the neuroprotective effects of these agents and their effect on antitumor activity, determined by relative survival.

Amifostine [Ethyol®, WR-2721: US Bioscience]

Amifostine (*S*-2(3-aminopropylamino)-ethylphosphorothioic acid) is an organic thiophosphate compound. It is a pro-drug in which the thiol group is masked by a phosphate group. Desphophorylation of amifostine by the plasma membrane-bound enzyme alkaline phosphatase forms the active protecting agent WR-1065, a non-protein bound free sulphydryl compound. Inside the cell, WR-1065 is further metabolized to mixed disulphides and sulfite products.[109]

Mode of action. WR-1065 is thought to protect normal tissue from damage by acting as a scavenger of oxygen free radicals[78,110] and by donating hydrogens to repair damaged target molecules.[77] Results of *in vitro* studies suggest that amifostine protection against cisplatin-induced toxicity results from prevention of cisplatin-induced cellular damage, rather than the reversal of damage.[80,81] WR-1065 prevented cisplatin–DNA adduct formation in a dose related manner *in vitro*, most likely the result of direct inactivation of cisplatin before hydrolysis can occur and inactivation of the reactive cisplatin aqua species before it can bind to DNA. To lesser extent, post-incubation with WR-1065 reversed pre-formed cisplatin–DNA adducts *in vitro*, possibly as a result of increased enzymatic repair resulting from WR1065-induced conformational changes in the DNA.[111] However, the kinetics of interaction of WR-1065 with cisplatin *in vitro* and the rapid clearance of cisplatin from the circulation by renal excretion and protein binding suggest that amifostine and its metabolites will not noticeably inactivate cisplatin in the circulation when amifostine is administered prior to or at the same time as cisplatin.[112] Confirming the results obtained *in vitro*, administration of amifostine (200 mg kg) prior to cisplatin did not reduce antitumor activity in nude mice bearing well-established OVCAR-3 (human ovarian cancer) xenografts.[113] In the same model, amifostine pre-treatment, but not post-treatment, greatly reduced cisplatin-induced nephrotoxicity, allowing a 2-fold escalation of the cisplatin dose.

Amifostine selectivity protects normal tissues. Tissue distribution studies with radiolabeled amifostine showed that the concentration of active thiol in normal tissues was 10- to 100-fold higher than in solid tumor tissues in Fisher rats.[114] This observation was subsequently confirmed in murine tumor models.[115–117] This differential uptake may reflect differences in the tissue microenvironment.[118] Since the biologic activity of alkaline phosphatase tends to be lower in malignant tissues than in normal tissues,[119,120] and since cellular uptake of amifostine is preceded by dephosphorylation to the active metabolite,[121,122] the low uptake of amifostine into tumor cells may reflect a reduced conversion to the active metabolite. In addition, uptake of amifostine is highly dependent on pH in the range of 6.6–8.2—preclinical studies predict a more than 2-fold decrease in the rate of uptake as the pH is decreased by 0.3 units[121]—and tumor cells tend to have a lower pH due to their predominantly anaerobic metabolism.

In vitro studies failed to show a selective effect of amifostine on cisplatin-induced toxicity. When co-incubated or administered prior to cisplatin, the thiol metabolite WR-1065, but not the prodrug amifostine, protected both OVCAR-3 (human ovarian cancer) and V79 (hamster lung fibroblast) cells against cisplatin-induced growth inhibition.[123] In contrast, another set of experiments showed that co-incubation with either amifostine or WR-1065 had no effect on the growth inhibition caused by cisplatin and 5-fluorouracil in C26-10 (murine undifferentiated colon adenocarcinoma), HT-29 (human colon carcinoma) and UM-SCC-11B (human head and neck squamous cell carcinoma) cell lines. Also, a 15 min pre-incubation with amifostine or WR-1065 had no effect on cisplatin-induced growth inhibition of the A2780 (human ovarian cancer) and OVCAR3 (human ovarian cancer) cell lines.[124] However, the combination of cisplatin, 5-fluorouracil and amifostine resulted in an enhanced antitumor activity *in vivo* against the murine colon tumors Colon 26 and Colon 38 compared to 5-fluorouracil alone and to 5-fluorouracil combined with cisplatin. The increased efficacy was not correlated with enhanced inhibition of thymidylate synthase, the target enzyme for 5-fluorouracil.[124,125]

Preclinical studies. Administration of amifostine 30 min prior to cisplatin allowed a 2- to 3-fold increase in cisplatin dose before the occurrence of nephrotoxicity in BALB c mice[118,126] and a 1.7-fold increase in cisplatin dose before the occurrence of nephrotoxicity in Fisher 344 rats.[127] In both mice and rats, simultaneous treatment with cisplatin or

administration of amifostine after cisplatin had no protective effect. However, amifostine pretreatment did not affect cisplatin antitumor activity in nude mice bearing OVCAR-3 (human ovarian cancer) xenografts,[113] nude mice bearing MCa-11 (murine mammary carcinoma) tumors,[126] or rats bearing DMBA-14 or 3M2N (rat mammary carcinoma) tumors.[126] Addition of amifostine also protected against cisplatin-induced neuropathy in cultured chick dorsal root ganglion[128] and the central nervous system of the snail.[129]

Clinical studies. In some human studies amifostine has partially protected against cisplatin-induced neuropathy and nephrotoxicity. In a phase I/II trial, patients receiving amifostine (740 mg/m^2, i.v.) 15 min prior to cisplatin (60–150 mg/m^2) had a lower incidence of nephrotoxicity [serum creatinine > 1.5 mg/dl in 10% (10/97) of courses with 120 mg/m^2 cisplatin] and peripheral nerve dysfunction (mild to moderate peripheral neuropathies observed in seven patients after a median cumulative platinum dose of 870 mg/m^2) than reported for historical controls.[130] A prospective study of patients receiving various cisplatin-based chemotherapy regimens found that patients receiving cisplatin (120 mg/m^2 q4w) with amifostine pretreatment (740 mg/m^2) had a significantly lower incidence of neuropathy [25 (7/28) versus 49% (34/69), $p < 0.05$] and a higher cumulative mean cisplatin dose at onset of neuropathy [635 versus 383 mg/m^2, $p < 0.005$] than patients treated without amifostine.[131]

In a multicenter phase III trial, patients with advanced epithelial ovarian cancer were randomized to receive six cycles of cisplatin (100 mg/m^2) plus cyclophosphamide (1000 mg/m^2), with or without amifostine pretreatment (910 mg/m^2) (Table 2). Interim analysis of the first 121 patients showed that amisfostine pretreatment was associated with moderate but significant reductions in cisplatin-induced neurotoxicity (19 versus 32%, $p = 0.022$), tinnitus (11 versus 26%, $p = 0.03$) and nephrotoxicity (3 versus 15%, $p = 0.040$).[132] Analysis of the full 200 patients (98 ACP and 102 CP) showed equivalent pathological response rates and median survival (34 months for ACP and 36 months for CP) with a median follow-up of 40 months,[133] suggesting that amifostine had no adverse effect on the antitumor activity of chemotherapy.

In contrast, no neuroprotective effect of amifostine was found in other phase II trials. In patients with recurrent/metastatic head and neck cancer receiving amifostine (740 or 910 mg/m^2 i.v.) prior to high dose cisplatin (120 mg/m^2) and 5-fluorouracil infusion, the incidence of cisplatin-related toxicities was similar to that expected with high dose cisplatin and 5-fluorouracil alone. The 32% (8/25) objective response rate reported in this trial also was similar to that obtained in previous studies of cisplatin/fluorouracil combination chemotherapy.[134] In patients with metastatic non-small cell lung cancer receiving

Table 2. Results of a multicenter phase III trial in patients with advanced ovarian cancer receiving cyclophosphamide (C) and cisplantin (P) ±amifostine (A)[131,133]

	A + CP	CP	*p* value
Adverse effects			
neurotoxicity			
(≥ grade II/III)	19% (12/63)	32% (18/58)	0.022
(> cumulative dose of cisplatin at onset of grade II/III)	502 mg/m^2	429 mg/m^2	0.078
tinnitus	11% (7/63)	26% (15/58)	0.031
nephrotoxicity (creatinine >1.5 mg/dl by day 25)	5% (5/98)	15% (15/102)	0.023
neutropenia (< 1500/mm^3 at day 25)	41% (40/98)	65% (66/102)	0.027
thrombocytopenia (< 50 000/mm^3 in any cycle)	3% (2/63)	13% (8/58)	0.056
hospitalization for neutropenia-related fever or sepsis	8% (5/63)	28% (16/58)	0.004
(mean duration of hospitalization)	5.2 da	8.6 da	0.022
(total days of hospitalization)	70 da	202 da	0.027
Efficacy			
pathological complete responses	36% (11/31)	29% (10/34)	NS
median survival (40 months follow-up)	34 months	36 months	NS

amifostine (910 mg/m^2) prior to cisplatin (120 mg/m^2) every 28 days plus vinblastine (5 mg/m^2) weekly, grade 3 neuropathy was observed in 27% (3/11) of patients at cumulative cisplatin doses of 600, 480 and 432 mg/m^2; grade 3 ototoxicity was observed in one patient at a cumulative cisplatin dose of 120 mg/m^2; and grade 3 nephrotoxicity was ob served in 18% (2/11) of patients.[135] Similarly, in patients with advanced cervical cancer receiving amifostine (340–910 mg/m^2/day) prior to cisplatin (20 mg/m^2/day × 5d) every 3 weeks in combination with radiation therapy, the incidence of cisplatin-related toxicities was similar to that expected with cisplatin alone. Audiometric studies detected ototoxicity in 44% (4/9) of patients tested, 25% (5/20) of patients developed severe nephrotoxicity and 10% (2/20) developed severe neurotoxicity.[136]

Several phase II studies have examined the role of amifostine with cisplatin in the treatment of metastatic melanoma. In a study conducted at the University of Pennsylvania,[68] objective responses were observed in 53% (19/36) of patients treated with cisplatin (120–150 mg/m^2 q4w) preceded by amifostine (740 mg/m^2). Peripheral neuropathy developed in 25% (9/36) of patients after a median cumulative cisplatin dose of 670 mg/m^2 and nephrotoxicity developed in 5%. In a similar study conducted at the Istitut Gustave-Roussey in France,[137] objective responses were observed in 35% (7/20) of evaluable patients treated with cisplatin (120 mg/m^2 q4w) preceded by amifostine (910 mg/m^2). Ototoxicity was observed in 30% (6/20) of patients, peripheral neuropathy in 10% (2/20) and nephrotoxicity in 15% (3/20). In a third study conducted at Yale University,[138] no objective responses were observed after six patients with metastatic melanoma were treated with cisplatin (100 mg/m^2 on days 1 and 8 every 4 weeks) preceded by amifostine (740 mg/m^2). All six patients developed some degree of ototoxicity based on serial audiograms and two patients developed nephrotoxicity.

The possibility of amifostine enhancement of cisplatin activity has been raised by preliminary results of a phase II study performed at the University of Wisconsin. Investigators reported a 74% (14/19) objective response rate and 17 month median survival in patients with stage III non-small cell lung cancer treated with amifostine (740–910 mg/m^2) prior to cisplatin (120 mg/m^2) on days 1 and 29 plus vinblastine (5 mg/m^2) weekly, followed by thoracic radiation and amifostine.[139,140] In another phase II study, the same group reported an 80% (8/10) objective response rate in patients with metastatic non-small lung cancer treated with amifostine (910 mg/m^2) prior to cisplatin (120 mg/m^2) every 28 days plus vinblastine (5 mg/m^2) weekly, with vigorous antiemetic and hydration support.[135] The effect observed in clinical trials was confirmed in nude mice bearing A549 subcutaneous tumors and treated with either amifostine (200 mg/kg) alone, combination chemotherapy with cisplatin (7.5 mg/kg) and vinblastine (50 μg/kg), or amifostine followed by cisplatin/vinblastine. Although amifostine alone had no effect on tumor growth, the addition of amifostine to cisplatin/vinblastine enhanced tumor inhibition.[141]

Amifostine is generally well tolerated, with transient side effects including mild to moderate nausea and vomiting (55% of courses, with limited use of antiemetics), a flushed feeling toward the end of the infusion (25–35% of patients), episodic sneezing (35%), mild somnolence (6%) and a metallic taste during infusion.[68,130,142] However, amifostine has been associated with transient hypotension which necessitates close monitoring during the 15 min infusion. Though minor decreases in blood pressure are quite common, up to 5% of patients develop a significant decrease in blood pressure, defined as >20 mm Hg decrease in systolic blood pressure for over 5 min or symptomatic hypotension, which necessitates discontinuation of treatment. Hypotension usually occurs at the end of the infusion and is reversed with discontinuation of amifostine, administration of saline and placing the patient in the Trendelenburg position. Patients with head and neck, esophageal and non-small cell lung cancers, or with prior neck irradiation or hypocalcemia are at higher risk of amifostine-induced hypotension.[143] Transient, asymptomatic hypocalcemia also has been reported.

Diethyldithiocarbamate (DDTC)

DDTC is an alkyl dithiocarbamate compound which is used as an antidote for acute nickel and cadmium poisoning. In animal models, administration of DDTC 2–3 h after cisplatin reduced the toxic side effects of cisplatin without altering its antitumor effects.[144–146] Observations that DDTC *in vitro* could reactivate cisplatin-inactivated renal enzymes but could not reverse cisplatin–DNA interactions supported the hypothesis that DDTC is able to reverse toxic cisplatin–protein interactions but not the cisplatin–DNA adducts responsible for anti-tumor activity.[147] However, clinical trials of DDTC (4 g/m^2) as a chemoprotector from the toxic effects of cisplatin (120–200

mg/m^2)[148–151] and carboplatin (800 mg/m^2)[152] were stopped due to severe but reversible DDTC-related autonomic hyper-activity. Only one of the trials showed substantial protection from nephrotoxicity with DDTC[148] and none of the studies showed a significant reduction in cisplatin-induced neuropathy or ototoxicity or carboplatin-induced myelosuppression.

Fosfomycin (Bristol-Myers Squibb)

Fosfomycin (1,2-epoxylpropylphosphonic acid) is a phosphonic acid antibiotic that inhibits phosphoenolpyruvate transferase, an enzyme essential in the final step of bacterial cell wall snythesis. The reactive sites on the phosphoenolpyruvate transferase are a nucleophilic sulfur of a cysteine residue and a proton donor. The presumed reaction between fosphomycin and phosphoenolpyruvate is a sulfhydryl addition across the C(2)–O(1) bond, analogous with the assumed sulfhydryl addition across the C(2)=C(3) double bond of phosphoenolpyruvate in the bacterial cell wall.[153] In both experimental models and humans, co-administration of fosfomycin protected against aminoglycoside-induced ototoxicity with a concomitant reduction in histological damage in the inner ear.[97,98]

Recent studies have shown that fosfomycin has a significant protective effect when co-administered with cisplatin at a known therapeutic tumoricidal dose for cisplatin in experimental animal models.[97,99,101,102] In the Fisher rat, 10 consecutive days of cisplatin (1 mg/kg qd) induces 100% outer hair cell loss in the basal turn of the organ of Corti. Co-administration of fosfomycin significantly reduced the outer hair cell loss (54 versus 100%).[97] In the albino guinea pig model, twelve consecutive days of cisplatin (1 mg/kg qd) induces clinical deafness (loss of Preyer's pinna reflex) and greater than 50% total outer hair cell loss in the basal turn of the organ of Corti with at least 20 dB threshold elevation at 6 and 15 kHz. Co-administration of fosfomycin (160 or 320 mg/kg qd) significantly reduced the cisplatin-induced outer hair cell loss and brainstem auditory evoked response threshold shifts at 2 and 6 kHz.[101,102]

Fosfomycin also has shown a protective effect against cisplatin-induced nephrotoxicity in humans.[154] In patients with lung cancer, treatment with fosfomycin (2 g bid days 1–4) following cisplatin (50 mg/m^2 on day 1) prevented an increase in urinary levels of *N*-acetyl-β-D-glucosaminidase, a marker of proximal tubular damage.

Glutathione (Boehringer Mannheim)

The tripeptide glutathione (γ-Glu-Cys–Gly) is the most abundant intracellular thiol. Glutathione plays a role in the detoxification of electrophilic drugs and metabolites and active oxygen formed during drug metabolism. Exogenously administered glutathione accumulates in the kidney,[155] an organ containing high levels of the membrane-bound enzyme γ-glutamyl transpeptidase involved in intracellular uptake of glutathione. As a result, glutathione was initially developed as a selective protector of cisplatin-induced nephrotoxicity.[156] However, peripheral nerves also contain high levels of γ-glutamyl transpeptidase,[157] suggesting that glutathione might also protect against cisplatin-induced neuropathy.

In a rat model for cisplatin-induced neuropathy, biweekly injections of cisplatin (1 mg/kg/dose, i.p.) for 10 weeks induced a sensory neuropathy characterized by a decrease in the H-reflex related sensory nerve conduction velocity, which is dependent on the thickest myelinated sensory nerve fibers. Co-administration of glutathione (500 mg/kg, i.v.) prevented the cisplatin-induced neuropathy in this model, even when the cisplatin dose-intensity was doubled.[158] In rats implanted with Walker/A mammary carcinoma, adminstration of glutathione (30 mg/kg) 15 min prior to cisplatin (1.5 mg/kg) did not affect the anti-tumor activity of cisplatin.[158]

Several phase II clinical studies in patients with advanced ovarian cancer indicate that pretreatment with glutathione has no adverse impact on the response rates observed for cisplatin-based chemotherapy.[159–163] A pharmacokinetic study showed that glutathione pretreatment does not influence cisplatin pharmacokinetics and does not change the bound fraction of cisplatin in plasma.[164] In a phase II study, patients with advanced ovarian cancer treatment with glutathione (2500 mg, i.v.) prior to high dose cisplatin (40 mg/m^2/day × 4d) and cyclophosphamide (600 mg/m^2) every 3–4 weeks for five courses were able to tolerate higher cumulative doses of cisplatin with a lower incidence of neurotoxicity than previously reported for the cisplatin/cyclophosphamide regimen. Analysis of toxicity data for 79 patients showed nephrotoxicity in 8% (6/79) of patients, mild hearing loss in 6% (5/79), ototoxicity defined by audiogram abnormalities in 32% (25/79) and peripheral neuropathy in 56% (44/79; severe in 3/79) after a median cumulative cisplatin dose of 800 mg/m^2.[165,166] In a phase II comparative study, patients with advanced ovarian cancer were randomly assigned to treatment with cisplatin (50 mg/m^2 weekly for 9 weeks) alone or

with glutathione pretreatment (2500 mg, i.v.). Neurophysiologic examinations conducted before and immediately after chemotherapy showed no effect on the sensory pathway with either regimen.[159]

In a double-blind randomized trial, 50 patients with advanced gastric cancer received reduced glutathione (1.5 g/m^2, i.v., 15 min before each cisplatin dose and 600 mg intramuscularly on the successive 4 days) or placebo in a weekly combination chemotherapy regimen with cisplatin, epirubicin, 5-fluorouracil and 6S-leucovorin. Compared with the placebo group after 9 and 15 weeks of therapy, patients receiving glutathione showed less reduction in median, ulnar and sural sensory nerve conduction, and an increased response rate (76 versus 56% overall response rate; 20 versus 12% CRs).[167]

Lazaroid (U74006F: Upjohn)

Lazoroids are non-glucocorticoid 21-amino steroid derivatives which inhibit lipid peroxidation and scavenge oxygen radicals. The Lazaroid U74006F has been found effective in treating experimental animal models of spinal cord injury and spinal trauma,[104,105] concussive head injury,[106] cerebral ischemia,[107] and ischemia-induced cochlear injury.[108] Co-administration of U74006F (10 mg/kg qd, p.o.) significantly reduced cisplatin-induced ototoxicity in an albino guinea pig model.[102]

Nerve growth factor (NGF) (Genentech; Synergen/Syntex)

NGF is a polypeptide growth factor necessary for the development of dorsal root ganglion sensory neurons and for the maintenance of normal ganglion function in adults. In a mouse model for cisplatin-induced neuropathy, weekly injections of cisplatin (10 mg/kg, i.p.) for eight consecutive weeks induced a sensory neuropathy characterized by a pronounced inability to maintain balance in the absence of visual cues, a small but significant elevation of thermal pain sensation threshold, a significant prolongation of sensory conduction latencies in the distant caudal nerve and a significant reduction in levels of calcitonin gene-related peptide per ganglion.[94,168] Co-administration of NGF (5 µg g three times a week) prevented the cisplatin-induced neuropathy in this model.[94]

Org2766 (Organon)

Org2766 (L-methionyl sulfone-L-glutamyl-L-histidyl-L-phenylalanyl-D-lysyl-L-phenylalanine) is an ACTH(4–9) analog without corticotropic or melanotropic activity. In an experimental model of sciatic nerve crush lesion, neurotrophic fragments and analogs of melanocortins, including Org2766, were able to restore nerve function over a period of several months when treatment was initiated within a short period after inducing the lesion.[82–86] Histological and functional studies suggest that melanocortins and ACTH analogs do not enhance the rate of nerve outgrowth, but rather increase the number of newly formed nerve sprouts at the site of the lesion,[83,85,169] perhaps by mimicking or amplifying an endogenous signal that operates early in the regenerative response of the damaged neuron.[90,91] At the cellular level, biotinylated Org2766 bound only to cells with neuronal characteristics in primary cultues of rat spinal cord and dosal root ganglion and to neuronally differentiated human neuroblastoma cells.[170]

In a rat model for cisplatin-induced neuropathy, biweekly injections of cisplatin (1 mg/kg/dose, i.p.) for at least 6 weeks induced a sensory neuropathy characterized by a decrease in H-reflex related sensory nerve conduction velocity, which is dependent on the thickest myelinated sensory nerve fibers. Co-administration of Org2766 (75 µg/kg every other day) prevented the cisplatin-induced neuropathy in this model,[87,88,171] even when the cisplatin dose intensity was doubled.[89] In full grown adult rats, co-administration of Org2766 counteracted a cisplatin-induced slowing of sensory nerve conduction velocity. However, Org2766 was not able to enhance recovery from an existing cisplatin neuropathy.[172] In rat embryo dorsal root ganglion cultures, Org2766 partially prevented cisplatin inhibition of NGF-induced neurite outgrowth but did not prevent cisplatin killing of rapidly dividing Schwann cells and fibroblasts. Org2766 alone had no neurotrophic effect for dorsal root ganglion outgrowth.[57]

Results of experimental animal models stimulated human clinical studies. In a randomized, double-blind, placebo-controlled study in women with advanced ovarian cancer, Org2766 (0.25 or 1.0 mg/m^2, s.c.) or placebo was administered before and after each dose of displatin in combination chemotherapy with cisplatin (75 mg/m^2) and cyclophosphamide (750 mg m^2) every 3 weeks.[92] Compared with the placebo group after six cycles of therapy, women receiving high-dose Org2766 showed significantly less change in the mean threshold value for vibration perception (0.88 versus 5.87, $p < 0.005$) and reported significantly fewer neurological clinical symptoms. All treatment groups showed similar overall response rates [60% (9 15) CRs in the placebo group versus

63% (5/8) CRs in the high-dose Org2766 group]; response rates were comparable with those reported previously for the chemotherapeutic regimen.[173–175] No adverse reactions other than those clearly related to chemotherapy (nausea, vomiting, diarrhea and impairment of renal function) were reported in any of the treatment groups. However, these results were not confirmed in a double-blind, randomized study in which 131 women with advanced ovarian cancer were given either Org2766 (2 mg, s.c.) or placebo before and after each dose of cisplatin in combination chemotherapy with cisplatin (75 mg/m^2) and cyclophosphamide (750 mg/m^2) every 3 weeks.[93] After six cycles of chemotherapy, the Org2766 and placebo groups showed equivalent vibration perception threshold values, paresthesia-free survival and clinical signs and symptoms.

Sodium thiosulfate

Sodium thiosulfate is a thiol compound used as an antidote for cyanide poisoning. *In vitro* and *in vivo* models show that sodium thiosulfate reacts with cisplatin to form covalently bound complexes.[176–178] In rabbits, sodium thiosulfate neutralized cisplatin in plasma by acting as a competitive antagonist.[179] However, sodium thiosulfate rapidly distributes into the extracellular space following intravenous administration,[180,181] so it is unlikely that it can interact with intracellular species of cisplatin.

Preliminary clinical studies indicated that concurrent administration of sodium thiosulfate and intraperitoneal cisplatin reduced the incidence of nephrotoxicity,[182,183] though hypomagnesemia associated with renal tubular dysfunction was reported. However, concurrent administration of sodium thiosulfate and intravenous cisplatin did not appear to protect patients from cisplatin-induced nephrotoxicity, peripheral neuropathy, ototoxicity, emesis or bone marrow suppression[184,185] and is likely to reduce cisplatin's anticancer activity.

Summary

Early clinical data suggests that several agents—including amifostine and other experimental agents—may be effective in reducing the incidence and severity of cisplatin-induced neurotoxicity without adversely affecting antitumor efficacy, reflected by overall response rates. Completion of ongoing, prospective, randomized clinical studies will clarify the neuroprotective effects of these agents and their effect on antitumor efficacy, determined by relative survival rates. Should these studies confirm the preliminary results, a neuroprotective agent would significantly enhance the clinical effectiveness of cisplatin, enabling dose intensive therapy and greater cumulative doses to be delivered to more patients with sensitive tumors.

Based on recently reported results of a multicenter phase III trial,[133,134] amifostine is likely to be the first neuroprotective agent used widely to enhance the clinical effectiveness of cisplatin. In women with advanced ovarian cancer receiving combination chemotherapy with cisplatin plus cyclophosphamide, amifostine pretreatment was associated with moderate but significant reductions in cisplatin-associated peripheral neuropathy, tinnitus and nephrotoxicity, while achieving equivalent pathological response rates and median survival.

Preclinical data suggest that several additional agents, especially the neurotrophic factors NGF, IGF-1 and NT-3, merit further investigation. Nerve growth factor is the only agent reported to prevent, rather than partially protect, cisplatin-induced neuropathy in an experimental model.

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