REVIEW ARTICLE

Drugs 1999 Mar; 57 (3): 293-308
0012-6667/99/0003-0293/$16.00/0

Chemoprotectants

A Review of their Clinical Pharmacology and Therapeutic Efficacy

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Abstract

Dose-limiting toxicity secondary to antineoplastic chemotherapy is due to the inability of cytotoxic drugs to differentiate between normal and malignant cells. The consequences of this may include impairment of patient quality of life, because of toxicity, and reduced tumour control because of the inability to deliver adequate dose-intensive therapy against the cancer. Specific examples of toxicity against normal tissues include cisplatin-related neurotoxicity and nephrotoxicity, myelotoxicity secondary to treatment with alkylating agents and carboplatin, oxazaphosphorine-induced haemorrhagic cystitis, and cumulative dose-related cardiac toxicity secondary to anthracycline treatment.

Chemoprotectants have been developed as a means of ameliorating the toxicity associated with cytotoxic agents by providing site-specific protection for normal tissues, without compromising antitumour efficacy. Clinical trials with toxicity protectors must include sufficient dose-limiting events for study, and assessment of both toxicity (allowing for measurement of efficacy of protection) and antitumour effect. Several chemoprotective compounds have now been extensively investigated, including dexrazoxane, amifostine, glutathione, mesna and ORG 2766.

Dexrazoxane appears to complex with metal co-factors including iron, to reduce the incidence of anthracycline-induced cardiotoxicity, allowing the delivery of higher cumulative doses of anthracyclines without the expected consequence of cardiomyopathy. Numerous studies have demonstrated that sulfur-containing nucleophiles, including amifostine, glutathione, and mesna can specifically bind cisplatin- or alkylating agent–generated free radicals or alkylating agent metabolites to reduce the incidence of cisplatin-associated neurotoxicity and nephrotoxicity, or alkylating agent-associated myelosuppression and urothelial toxicity. These studies, in the majority of instances, have not revealed any evidence of reduction in antitumour efficacy.

Further randomised trials are required to identify the optimal role of chemoprotectants when used alone or in combination with other toxicity modifiers including haemopoietic growth factors.

Significant advances in the reduction of antineoplastic chemotherapy-associated toxicity have occurred in recent years. These include the development of analogues of established cytotoxic agents with improved toxicity profiles and comparable antitumour efficacy, improvement in control of chemotherapy-associated emesis with the use of serotonin ($5HT_3$) antagonists in combination with corticosteroids, alteration in schedules of cytotoxic drug administration, and the use of haemopoietic colony-stimulating factors to alleviate treatment-related myelosuppression and its consequences.

The delivery of adequate doses of chemotherapy is often compromised by the narrow therapeutic index of cytotoxic agents, owing principally to the inability of these drugs to differentiate between malignant and normal cell populations. Damage to normal cells results in dose-limiting toxicity, potentially compromising patient quality of life, and the delivery of adequate dose-intensive treatment.

The concept of site-specific inactivation of chemotherapy drugs with chemoprotective agents has now been extensively explored in both preclinical and clinical studies. The aim of chemoprotective agents is to improve the therapeutic ratio of the cytotoxic drug by reducing potential dose-limiting toxicity to normal tissue. By definition, chemoprotectants must not compromise the antitumour efficacy of the chemotherapy agent, and they should not be associated with additional toxicity that might otherwise interfere with the delivery of adequate chemotherapy.

The first chemoprotectant to be used was folinic acid (calcium folinate; leucovorin), designed to overcome methotrexate-induced toxicity. With the development in understanding of the mechanism of damage to normal tissue caused by cisplatin, alkylating agents and anthracyclines, specific agents have been formulated and examined in clinical studies, with the aim of reducing observed tissue toxicity to normal tissue. In particular, sulfur-containing nucleophiles and metal-chelating agents have been demonstrated to be active chemoprotective compounds in numerous trials and are the subject of this review.

1. Nucleophilic Sulfur Compounds

The efficacy and toxicity of alkylating agents, including cisplatin, are mediated by the formation of highly reactive electrophilic intermediates.[1] These intermediates bind to nucleophilic targets, such as the N7 position of guanine in DNA, and form covalent bonds. Nucleophilic sulfur compounds such as glutathione can be reduced to provide an alternative nucleophilic target and can thereby inactivate these reactive intermediates. The unreduced nucleophilic sulphur compounds can also bind other reactive species, such as oxygen free radicals, which mediate damage to normal tissue following exposure to a variety of agents including irradiation. This has given rise to interest

in this class of compounds as radioprotective and chemoprotective agents.

Sodium thiosulphate is one such agent. However, when given systemically it inactivates cisplatin in the circulation.[2] Its potential is limited to systemic protection after local (e.g. intraperitoneal or intra-arterial) therapy. Therefore, interest has focused on those nucleophilic sulfur agents that undergo selective activation or uptake in normal tissue. The 2 agents that have reached clinical trials are amifostine and reduced glutathione.

1.1 Amifostine

Amifostine (WR-2721) is a nucleophilic sulfur prodrug which is dephosphorylated to the active agent WR-1065 by membrane alkaline phosphatase. Numerous preclinical studies have shown amifostine to be a selective chemoprotective agent (reviewed by Capizzi[3]). After administration, amifostine is rapidly and extensively taken up into normal tissues. A much lower (1 : 50 to 1 : 100) amount of amifostine accumulates in tumour tissue. The disparity in uptake is thought to relate to differences in membrane alkaline phosphatase activity and pH between tumour and normal tissue.[4] Once inside normal cells, WR-1065 binds to and inactivates oxygen free radicals, activated platinum species and alkylating agents. In a variety of models amifostine protects normal tissues (bone marrow, peripheral nerve, heart and kidney) against the cytotoxic effects of alkylating agents, platinum compounds, anthracyclines, taxanes and irradiation without compromising antitumour cytotoxicity.[3]

The recent finding that amifostine can stimulate bone marrow precursors in patients with myelodysplasia raises the possibility that some of the protective effect against bone marrow toxicity is the result of an intrinsic stimulatory effect on the bone marrow.[5] The ability of amifostine to reduce the mutagenic effects of alkylating agents is particularly important in view of the increasing recognition of second malignancies in cured patients.[6]

1.1.1 Phase I and Pharmacokinetic Trials

Phase I trials of amifostine administered as a single intravenous dose of up to 1330 mg/m^2 did not identify a maximum tolerated dose.[7,8] Subsequent studies have generally utilised a recommended adult dose of 910 mg/m^2 administered as a 15-minute infusion approximately 30 minutes before initiation of each cycle of chemotherapy.

Amifostine pharmacokinetics have been recently summarised by Van der Vijgh and Korst.[9] Amifostine is rapidly cleared from plasma by a biphasic decay pattern with a distribution half-life of 0.88 min and an elimination half-life of 8.8 min. The initial volume of distribution approximates intravascular volume. The majority of the administered dose is retained in the tissues where it is converted by alkaline phosphatase to WR-1065. The active form, WR-1065, is further oxidised to WR-33278 (the symmetric disulphide of WR-1065) and also to mixed disulphides. The total urinary excretion of amifostine and its metabolites equalled 6% of the administered dose. Because of the short half-life of amifostine, chemoprotection for cytotoxics that have prolonged plasma exposure (e.g. carboplatin) may require supplemental doses of amifostine at later time points.

Hypotension is the major toxicity described and requires careful monitoring.[10-13] Patients receiving antihypertensive agents should have them omitted for 24 hours before treatment with amifostine, and the drug is contraindicated in patients who are likely to become compromised by hypotension. Guidelines are published for recommended management of amifostine-induced hypotension.[14] Patients unable to tolerate the full dose may tolerate a reduced dose on subsequent cycles. Nausea and vomiting, sneezing, dizziness, flushing, metallic taste, anxiety and urinary retention are also reported. Hypocalcaemia may occur, particularly with daily administration schedules. This is caused by inhibition of parathyroid hormone activity and may require calcium supplementation.[15]

1.1.2 Cytotoxic Dose Escalation With Amifostine

One proposed use for amifostine is to allow delivery of cytotoxic agents at greater dose intensity than conventional regimens, with the primary aim being to improve the response to the cytotoxic therapy. There are no phase III studies of this strat-

egy. A phase I trial of amifostine and melphalan in heavily pretreated paediatric patients with cancer demonstrated some of the limitations of this approach.[10] Amifostine did not allow significant dose escalation of melphalan above the maximum tolerated dose of 35 mg/m^2 in this population.

Phase II trials in melanoma and non–small cell lung cancer have demonstrated that the addition of amifostine allows only modest increases in cisplatin dose intensity. In combination with amifostine 740 to 910 mg/m^2 before each cisplatin dose, Schiller et al.[11] were able to administer cisplatin 120 mg/m^2 every 4 weeks with vinblastine 5 mg/m^2 weekly to patients with metastatic non–small cell lung cancer. Glover et al.[12] treated 36 patients with metastatic melanoma with amifostine (740 mg/m^2) and single agent cisplatin. They were unable to escalate the dose of cisplatin to 150 mg/m^2 and eventually found the maximum tolerated dose to be 135 mg/m^2. In both trials toxicity was acceptable and the response rates of 64% (with a median survival of 17 months) for non–small cell lung cancer[11] and 53% for metastatic melanoma[12] were encouraging compared with results from other phase II trials. However, comparisons of response rates from phase II studies are potentially confounded by differences in patient selection.

1.1.3 Toxicity Prevention with Conventional Dose Regimens and Amifostine

The alternative strategy utilised is to add amifostine to conventional dose regimens of cytotoxic agents, with the primary aim of reducing toxicity and perhaps increasing delivered dose intensity of the chemotherapy.

Two early trials established that amifostine protects against haematological toxicity of single agent cyclophosphamide.[7,16] More recently amifostine has been used to protect normal bone marrow against the effects of purging with the alkylating agent 4-hydroperoxycyclophosphamide.[17] The effect of amifostine in combination with mitomycin was assessed by Poplin et al.[18] They performed a randomised comparison of mitomycin with or without amifostine 910 mg/m^2 in patients with advanced colorectal cancer. Amifostine reduced thrombocytopenia but both regimens were inactive.

The majority of recent trials have tested the efficacy of amifostine in conjunction with platinum-based regimens. Two studies have assessed the effect of amifostine on carboplatin toxicity. Betticher et al.[19] performed a randomised phase II trial comparing amifostine 910 mg/m^2 plus carboplatin 600 mg/m^2 versus carboplatin alone in patients with non–small cell lung cancer. Amifostine was given 20 minutes before and at 2 and 4 hours after carboplatin administration (total amifostine dose 2730 mg/m^2) There was no significant difference in neutrophil nadir or recovery between the 2 groups. The time to platelet recovery (13.5 *vs* 21 days, $p = 0.04$) and need for hospitalisation (0/20 *vs* 6/25 courses, $p = 0.06$) favoured the amifostine arm. Interpretation of this trial is limited by its small size ($n = 21$) and the confounding effect of dose reductions in the carboplatin alone arm (2/10 *vs* 0/11 patients).

A larger study was performed by Budd et al.[20] who randomised 55 patients with a variety of tumours to carboplatin 500 mg/m^2 with or without amifostine 910 mg/m^2 given 15 minutes before and 2 hours after carboplatin. (total amifostine dose 1820 mg/m^2). They found that the median platelet nadir for all cycles was higher in the amifostine arm (127 *vs* 88×10^9/L, $p = 0.023$) and there was no difference in the incidence of neutropenia. This study was not designed to allow meaningful comments about antitumour efficacy because of inclusion of patients with nonmeasurable disease and a variety of tumour types.

The best evidence for the chemoprotective efficacy of amifostine comes from an excellent, large randomised study by Kemp et al.[13] 242 patients with stage III and IV ovarian cancer were treated with cisplatin 100 mg/m^2 and cyclophosphamide 1000 mg/m^2 administered every 3 weeks. Patients were randomised to receive amifostine 910 mg/m^2 given as a 15-minute infusion before cyclophosphamide or no amifostine. Dose reductions were scheduled for haematological toxicity. Response was assessed by second look laparotomy in patients with clinical complete remissions. Toxicity and

Table I. Evaluation of amifostine as a chemoprotectant. Comparison of the toxicity and efficacy of cisplatin plus cyclophosphamide with and without amifostine in patients with ovarian cancer[13]

	Amifostine + cisplatin/cyclophosphamide (no. = 122)	Cisplatin/cyclophosphamide (no. = 120)	p value[a]
Toxicity			
Percentage of patients with neutropenia and fever/infection	10%	21%	0.019
Days in hospital	89	226	0.019
Creatinine level >1.5 mg/dl at day 22	5%	15%	0.014
40% reduction in creatinine clearance by last cycle	13%	30%	0.001
Tinnitus	9%	16%	0.108
Grade 2/3 neurotoxicity at last cycle	31%	42%	0.09[a]
Treatment-limiting renal, neurological or ototoxicity	10%	26%	0.001
Grade 3/4 nausea/vomiting	30%	23%	0.22
Efficacy (as assessed at second-look laparotomy)			
Pathological complete response	43.3%	36.5%	
Pathological complete response and clinical partial response	75%	65%	

a Chi squared recalculated for this parameter (not from original article).

efficacy data from this study are summarised in table I.

Significantly fewer patients discontinued treatment in the amifostine arm (9 *vs* 25%, p = 0.002).[13] Significant reductions ($p < 0.05$) were also seen in the incidence of neutropenia with fever (10 *vs* 21%), days in hospital (89 *vs* 226) and grade 2/3 neurotoxicity (31 *vs* 42%). The number of patients with a reduction in creatinine clearance of >40% (as calculated by the Cockcroft and Gault formula) was reduced from 30 to 13% (p = 0.001). Amifostine was generally well tolerated. Hypotension was common (61%), but usually transient. However, 17 patients did not complete the protocol because of hypotension. Most significantly, there was no compromise in the pathological response rate (amifostine 37% *vs* control 28%) or median survival time (31 months in both arms).

It is unclear whether increased dose intensity was achieved in the amifostine arm.[13] The investigators stated that the number of dose reductions in both groups was the same; however, the protocol specified dose reductions for haematological toxicity, which was greater in the control arm. This trial is very informative because it is a large randomised trial showing an improvement in clinically significant end-points without any apparent compromise in antitumour efficacy when amifostine is used. Haematological protection was sustained throughout 6 cycles of treatment but neurotoxicity remained a problem (31% neurotoxicity, predominantly grade 2). Although this is a reasonable sized study it was still designed to show a reduction in toxicity and was too small to detect small reductions or increases in therapeutic efficacy.

1.1.4 Drug Interactions

Amifostine is incompatible with cisplatin when administered by simulated Y site injection[21] and has been found to reduce binding of epirubicin to various plasma proteins.[22] Clearly, the potential for drug interactions with highly protein bound drugs needs to be investigated further. The most significant documented drug interaction thus far is between amifostine and carboplatin. Amifostine increases the area under the drug concentration-time curve (AUC) of carboplatin, which is associated with an increase in the terminal half-life.[9] This was attributed to a reduction in glomerular filtration rate as a result of the transient hypotension induced by amifostine. This change in AUC may explain the increase in antitumour efficacy seen with carboplatin in mice models when amifostine is administered.[3]

1.1.5 Conclusions

Amifostine is the most thoroughly evaluated of the newly available chemoprotective agents. There is a clear biological basis for its ability to selectively protect normal tissues. Phase II trials with amifostine have demonstrated the possibility of modest increases in dose intensity of cisplatin, with encouraging efficacy in melanoma and non–small cell lung cancer. The clinical significance of these modest increases in dose intensity needs to be evaluated in phase III trials. An adequately sized randomised trial in patients with ovarian cancer has demonstrated an improvement in the therapeutic index of cisplatin/cyclophosphamide chemotherapy when amifostine is administered. However protection is partial and neurotoxicity remains a problem.

Caution is required in extrapolating these results to other chemotherapy combinations or to more sensitive tumour types. There is no evidence from clinical trials that amifostine reduces the efficacy of cytotoxic chemotherapy and this is supported by reassuring preclinical data. However, it is important to remember that a small, but clinically significant, increase or decrease in survival cannot be excluded with current data. Future studies need to carefully evaluate pharmacokinetic interactions and explore the potential of amifostine in other combinations.

1.2 Glutathione

Glutathione is a natural thiol tripeptide involved in detoxification and protection from oxidant injury. The role of intracellular glutathione in determining sensitivity to cisplatin and other agents remains controversial. Extracellular glutathione is not normally taken up by cells except those expressing high levels of γ-glutamyl transpeptidase (γ-GT) activity, which includes kidney and neural tissue in the rat. Of some concern is the recent report that platinum complexes can upregulate γ-GT expression in some cell lines, which results in an increase in intracellular glutathione levels.[23] Studies in cell culture and animal models that have compared the cytotoxicity of cisplatin with or without added glutathione have shown no decrease in the tumour cell kill.[24] In fact, administration of extracellular glutathione is inhibitory to some ovarian cancer cell lines,[25] probably because of extracellular oxidation.[26]

Interest in glutathione initially arose from its potential to protect against cisplatin-induced nephrotoxicity. Several authors have shown that administration of glutathione reduces the incidence of renal dysfunction and neurotoxicity after cisplatin administration in the rat.[27,28] At present, it is not known whether increases in the tissue distribution and rate of elimination of cisplatin when given with glutathione contribute to the protective effect of glutathione.[29]

1.2.1 Clinical Trials

The encouraging protective effects of glutathione in preclinical evaluation led to a number of small phase II/phase III trials of glutathione with cisplatin-based chemotherapy. These trials demonstrated less than expected neuropathy and minimal nephropathy.[30,31]

There have been 4 small randomised phase II or phase III trials conducted, which have utilised different schedules of glutathione with variable results. Results from these trials are summarised in table II. Parnis et al.[32] gave glutathione 1.5 g/m^2 before a 2-hour infusion of cisplatin 40 mg/m^2/day for 2 or 3 days (total glutathione dose 3 to 4.5 g/m^2, total cisplatin dose 80 to 120 mg/m2) This trial was terminated after treatment of 22 patients because of unacceptable ototoxicity, presumed to be due to cisplatin. Two other randomised trials of patients (n = 54 and 33) with ovarian cancer utilised a 30-minute infusion of cisplatin 50 or 75 mg/m^2 (cumulative protocol dose 450 mg/m^2) alone or 15 minutes after pretreatment with glutathione 2.5g.[33,34] Both studies used multiple end-points and compared multiple subgroups making a definitive comment about neurotoxicity difficult. However both showed a trend to less neurotoxicity without an effect on response or other toxicity.

Another trial comprised 50 patients with advanced gastric cancer who received weekly cisplatin 40 mg/m^2 over 30 minutes, epirubicin, fluorouracil,

Table II. Randomised trials of glutathione (GSH) as a chemoprotectant for cisplatin-based regimens

Reference	No. of pts	Schedule[a]		Overview of results (GSH *vs* controls)
		cisplatin	GSH[b]	
Ovarian cancer				
Parnis et al.[32]	22	80-120 mg/m^2 (40 mg/m^2 for 2-3 days as 2h infusions)	3 to 4.5 g/m^2 total dose (i.e. 1.5 g/m^2/day for 2 to 3 days)	No difference in neuropathy between groups. Trial aborted because of unacceptable ototoxicity
Bogliun et al.[33]	54	50 mg/m^2 weekly or 75 mg/m^2 every 3 wks	2.5g	No difference in neuropathy between groups at wk 9, but trend to less neuropathy with GSH with more prolonged treatment. Response 19/27 *vs* 16/27
Colombo et al.[34]	33	50 mg/m^2 weekly	2.5g	Neuropathy at wk 9: 2/16 *vs* 4/15. Decrease in SAP: 26 *vs* 43%. Response 9/15 *vs* 12/16. Increase in administered cisplatin dose in GSH group
Gastric cancer				
Cascinu et al.[35]	50	40 mg/m^2 weekly[c]	1.5 g/m^2 + 600mg IM days 2-5[d]	Neuropathy at wk 9: 0/25 *vs* 9/25. Neuropathy at wk 15: 4/24 *vs* 16/18. Less deterioration evident in nerve conduction studies reduction in neurophysiology. Response 76 *vs* 52%

a All drugs given intravenously unless otherwise stated.

b Dose per cisplatin cycle.

c This regimen also included epirubicin, fluorouracil, 6S-leucovorin (folinic acid) and granulocyte colony-stimulating factor.

d Glutathione was given as an intravenous injection of 1.5 mg/m^2 before each cisplatin dose plus an intramuscular injection of 600 mg/m^2 on days 2 to 5.

IM = intramuscular; **pts** = patients; **SAP** = sural nerve action potential; **wk** = week.

6S-leucovorin (folinic acid) and granulocyte colony-stimulating factor for 9 weeks, with a further 6 weeks of treatment for patients who were responsive or had stable disease. Patients were randomised to receive placebo or glutathione administered by intravenous injection 1.5 g/m^2 prior to cisplatin, followed by glutathione 600mg on days 2 to 5 by intramuscular injection.[35]

In this trial, 7 of 25 patients in the placebo arm compared with 1 of 25 in the glutathione arm ($p = 0.04$) failed to complete the course of treatment, and there were slightly more treatment delays in the placebo arm.[35] Of the 7 withdrawals in the placebo arm, 6 were due to progressive disease. At week 9, clinical neuropathy was detected in 9 placebo patients compared with 0/25 in glutathione-treated patients. At week 15, neuropathy rates were 16/18 *vs* 4/24 respectively. Significant deteriorations in median, ulnar and sural nerve latency and action potential amplitude seen in the placebo arm were not found in the glutathione arm. Fewer transfusions were required in the glutathione arm, whereas other toxicities were equivalent. The response rate was 76% (95% confidence interval 60 to 92%) in the glutathione arm and 52% (95% confidence interval 33 to 71%) in the control arm. Complete response rates were 20 and 12%, respectively. For patients receiving glutathione, median survival time was 14 months compared with 10 months in the placebo arm.

This trial provides evidence for a protective role of glutathione. The reduction in toxicity achieved was clinically significant. These results need to be duplicated in adequately powered trials in other tumour types, ideally with pathological assessment of tumour response. The borderline or negative results from the other trials may relate to differences in schedules used or the small sample sizes. Although the optimal schedule has not been determined it would be reasonable to conduct further studies with a half-hour infusion of cisplatin with glutathione given as a 15-minute infusion before cisplatin and further doses given by intramuscular injection over 72 hours.

2. ORG 2766

ORG 2766 is an analogue of a corticotropin (ACTH) [4-9] fragment which in animal studies

has been shown to reduce peripheral nerve damage after a variety of insults including crush injury,[36] cisplatin[37] and paclitaxel (taxol) treatment.[38] The nonspecific nature of protection after ORG 2766 suggests that it acts directly to facilitate nerve repair, although the exact mechanism is unclear. The lack of an effect of ORG 2766 on tumour growth is not well documented.

There are 3 published randomised trials of ORG 2766 given in combination with platinum-based chemotherapy regimens. Interest in ORG 2766 was stimulated by a small double-blind placebo-controlled randomised trial (n = 55) in women with ovarian cancer receiving cisplatin 75 mg/m^2 and cyclophosphamide 750 mg/m^2 every 3 weeks.[39] Neurotoxicity was assessed by changes in vibration threshold. ORG 2766 was administered subcutaneously immediately prior to cisplatin and 24 hours later, at a dose of either 0.25 mg/m^2 or 1 mg/m^2. Patients in the 1 mg/m^2 group showed significantly lower thresholds of vibration perception after 4 and 6 cycles of treatment, indicating protection against cisplatin-induced neuropathy. However after exclusion of the 50% of patients in the low dose arm and the 30% of patients who were not evaluable, there were only 7 and 12 patients left for this comparison.

A similar randomised controlled trial has been performed in patients with testicular cancer or carcinoma of unknown primary origin who received cisplatin (20 mg/m^2 on days 1 to 5) with etoposide and either bleomycin or ifosfamide.[40] Patients were randomised to receive either subcutaneous ORG 2766 2 mg/day for 5 days or to placebo. Among patients who received 4 or more treatment cycles, those treated with ORG 2766 had a smaller increase in vibration threshold (0.45 to 1.85) than was seen in the placebo arm (0.35 to 4.03). The number of patients available for this analysis was again small (8 *vs* 12) due to a high proportion of patients who were either nonevaluable (13 of 55) or who did not receive 4 cycles of treatment.

Neither of these 2 trials demonstrated any adverse effects that were attributable to ORG 2766 and clinical response rates were similar with or without the drug.[39,40] However, late-onset neuropathy was subsequently observed in patients treated with ORG 2766 in 1 of these studies, suggesting ORG 2766 may merely delay neuropathy and that the benefit of treatment may have been overstated.[41] These trials also raised uncertainty as to the optimal dose of ORG 2766.

In view of the limitations of these studies, a large (n = 196) randomised double-blind placebo-controlled trial was performed in women with ovarian cancer who received cisplatin 75 to 100 mg/m^2 and cyclophosphamide 600 to 1000 mg/m^2 in 3- to 4-week intervals.[42] Women were randomised to receive either placebo or 2 or 4mg of ORG 2766 administered immediately before chemotherapy and 1 hour following the completion of post-chemotherapy hydration. Neuropathy was assessed by measurement of vibration threshold and patients were followed for 3 months following the cessation of chemotherapy. All groups were well matched for baseline prognostic factors, over 90% of patients were evaluable and over 75% of patients in all groups received a cumulative dose of cisplatin of >375 mg/m^2. There were no significant differences in vibration threshold between the 3 groups and, if anything, there was a trend towards worse neuropathy with treatment with ORG 2766.

This was a well performed study that addressed the methodological limitations of the previous studies. It therefore seems unlikely that ORG 2766 offers clinically significant neuroprotection when given with conventional dose cisplatin and cyclophosphamide.

3. Dexrazoxane

3.1 Mechanism of Action in Anthracyline-Induced Cardiotoxicity

The anthracycline antibiotics, including doxorubicin (adriamycin), daunorubicin and epirubicin, are among the most active anticancer agents against a wide range of solid and haemopoietic malignancies.[43] Mechanisms of cytotoxicity include intercalation of DNA[44] and inhibition of topoisomerase II.[45]

Anthracycline-induced cardiac toxicity can limit effective clinical use of the compounds. Cardiac toxicity may occur acutely with associated electrocardiogram (ECG) changes, arrhythmias or a myocarditis-pericarditis syndrome.[46] The most serious form is a chronic cumulative dose-related cardiomyopathy. The incidence of chronic congestive cardiac failure is 0.14% at total doxorubicin doses of <400 mg/m^2, increasing to 7% at 550 mg/m/^{2}, and to 18% at 700 mg/m^2.[47] Finally, recent studies have revealed a risk of late cardiac toxicity, including ventricular dysfunction and arrhythmias, occurring more than 1 year after exposure to anthracyclines.[48,49]

These studies raise the concerning issue that long term survivors of cancer who have received anthracycline-based chemotherapy may have an increased risk of cardiac morbidity and mortality as a consequence of their treatment. Lipshultz and colleagues[48] found that up to 65% of patients who received an anthracycline as treatment for acute leukaemia had evidence of cardiac dysfunction 15 years after treatment, which appeared to be progressive. Steinherz et al.[49] also demonstrated cardiac abnormalities in patients 4 to 10 years after completion of anthracycline therapy.

The cardiotoxic effects of anthracyclines appear to be associated with the generation of reactive oxygen species. The anthracycline structure contains a quinone group that undergoes electron reduction via nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reactions to produce a semi-quinone radical. Interaction of this semi-quinone with oxygen initiates a cascade of reactive oxygen species.[50-52] These reactive oxygen species react with lipids and other cellular constituents to cause damage to mitochondrial and cell membranes. By virtue of lower endogenous levels of antioxidant enzymes, the heart appears to be particularly susceptible to free radical damage.[53] Initial attempts at preventing anthracycline-induced cardiac damage focused on the administration of reactive oxygen species scavengers. However, studies with acetylcysteine[54] and tocopherol (vitamin E) [55] did not demonstrate any cardioprotective activity in humans.

A second pathway for reactive oxygen species production involves the formation of an anthracycline-iron complex. As has been shown for doxorubicin, anthracylines bind the ferric (Fe^{+++}) cation in the hydroquinone positions to form a complex which may undergo an internal redox reduction, generating an anthracycline-iron (Fe^{++}) free radical complex. Reaction of this molecule with oxygen results in formation of reactive oxygen species such as superoxide and anthracycline-iron (Fe^{+++}), with the latter being capable of undergoing further internal redox reduction.[56] In addition, the anthracycline-iron (Fe^{+++}) complex is a powerful oxidant itself, able to initiate lipid peroxidation in the absence of oxygen free radicals.[57]

With the recognition that the anthracycline-iron complex was also capable of generating reactive oxygen species, studies utilising metal chelators were initiated.

The most promising metal-chelating agents were found to be 2 drugs belonging to the bisdioxopiperazine family. Early studies[58] with razoxane (ICRF-159) demonstrated a cardioprotective effect during acute and chronic treatment with doxorubicin and daunorubicin in mice and hamsters, and a variety of other animal models.[59] Animal studies also provided important information in the timing of administration of razoxane, with the protective effect being maximal when the agent was given immediately prior to the anthracycline.[60] Dexrazoxane (ICRF-187) is the more water soluble (+)-enantiomer of razoxane, which can be administered parenterally. Preclinical animal studies also confirmed the cardioprotective activity of dexrazoxane.[61,62] These compounds undergo intracellular hydrolysis, forming a bidentate chelator. The chelator binds to intracellular iron, removing Fe^{++} from the anthracycline-iron complex, thereby preventing free radical generation.

3.2 Clinical Trials

Phase I studies of dexrazoxane, administered as a single agent, showed it to be well tolerated.[63,64]

Dose-limiting toxicity, in the form of leucopenia, occurred at intravenous doses >4000 mg/m^2, with no evidence of cumulative toxicity. Nonhaematological toxicity included mild nausea and vomiting, mild alopecia, transient elevations in serum hepatic transaminase levels and pain at the injection site.

The initial clinical trial of dexrazoxane in humans was conducted by Speyer et al.[65,66] In this randomised, non-crossover study in women with advanced breast cancer, patients were treated with FDC (fluorouracil 500 mg/m^2, doxorubicin 50 mg/m^2, cyclophosphamide 500 mg/m^2) administered every 3 weeks, either alone or preceded by dexrazoxane 1000 mg/m^2 (dose ratio of dexrazoxane to doxorubicin of 20 : 1). Dexrazoxane was given as a bolus intravenous injection 30 minutes prior to doxorubicin. End-points for cardiac toxicity assessment included clinical heart failure, left ventricular ejection fraction measured by radionuclide scan and pathological changes on endomyocardial biopsy.

Significant reductions in cardiac toxicity, as measured by the previously described end-points, were noted in the group treated with dexrazoxane (see table III).[65,66] There was no reduction in antitumour efficacy in the group receiving dexrazoxane. Although the incidence of myelosuppression was slightly higher in the dexrazoxane-treated group, this was not considered clinically significant.

Subsequent randomised studies have confirmed the cardioprotective effect of dexrazoxane. These include trials in patients with metastatic breast cancer,[67-70] small cell lung cancer,[71] and paediatric sarcoma.[72] In the paediatric study, patients given dexrazoxane and doxorubicin were less likely to develop subclinical cardiotoxicity than patients receiving doxorubicin alone (22 *vs* 67%, $p < 0.01$), and also received a higher median cumulative dose of doxorubicin ($p < 0.005$). Objective response rates between the 2 groups were equivalent, and there was no difference in event-free or overall survival. This study has important implications, yet to be confirmed, that dexrazoxane may reduce the incidence of late cardiac complications in long term survivors of childhood cancer treated with anthracycline-based chemotherapy.

The efficacy of dexrazoxane in reducing the risk of developing doxorubicin-associated cardiomyopathy in women with advanced breast cancer has been confirmed in a recently published

Table III. Randomised trial of the cardioprotective effect of dexrazoxane in patients with advanced breast cancer treated with doxorubicin-based chemotherapy[65,66]

	FDC	FDC + dexrazoxane	p value
Patient numbers	74	76	
Cardiac toxicity			
Clinical cardiac failure (no. of pts)	20	2	<0.001
Reduction in LVEF[a] (no. of pts)	32	5	<0.00001
Pathological changes on endomyocardial biopsy (score ≤2) [no. of pts]	6 (14)	0 (16)	<0.05
Median fall in LVEF (%)	15-16	1-3	<0.001
Doxorubicin dose intensity			
Median no. of cycles given	9	11	<0.001
Median cumulative doxorubicin dose (mg/m^2)	441	500	
Cumulative doxorubicin dose >1000 mg/m^2 (no. of pts)	0	11	NS
Efficacy			
Response rate (complete + partial) [no. of pts]	20	21	NS
Median months to disease progression	9.3	10.3	NS

a Study criteria for a reduction in LVEF were a decrease in LVEF to <0.45 or a decrease in baseline LVEF of 0.20 or more.

FDC = fluorouracil, doxorubicin + cyclophosphamide; **LVEF** = left ventricular ejection fraction; **NS** = not significant; **pts** = patients.

study.[69] The initial study treated patients with dexrazoxane to doxorubicin ratio of 20 : 1, but this was later reduced to a ratio of 10 : 1 because of concerns about possible additive toxicity.

In this placebo-controlled randomised trial, the hazard ratio (the increased risk of occurrence) for a cardiotoxic event was estimated to be 2.9 for placebo compared with the dexrazoxane group.[69] Of potential concern was the finding that the response rate for the dexrazoxane arm was significantly lower than for the placebo arm (47 *vs* 61%), although there was no significant difference in time to progression or overall survival between the 2 arms. A second follow-up study, reported in the same paper, confirmed the reduced incidence of cardiac events in the dexrazoxane group.[69] Furthermore, there was no difference in objective response rates (dexrazoxane 53.7%, placebo 49.3%) or progression free and overall survival between the 2 arms.

Following an interim analysis of the above studies, which confirmed the increased risk of cardiac toxicity in the placebo-treated group, the study was amended to include randomisation to placebo versus dexrazoxane for the first 6 cycles (cumulative doxorubicin dose of 300 mg/m^2), followed by open-label therapy with dexrazoxane from the seventh cycle onwards in both arms.[73] In an analysis of patients treated beyond course 6 with dexrazoxane (i.e. those who received placebo for the first 6 cycles and then received open-label dexrazoxane) compared with the earlier cohort who were treated with placebo for the entire study duration, the hazard ratio for a cardiotoxic event was 3.5 for the placebo arm. There was no difference in response parameters or survival between the 2 groups.

Although the analysis suggested that the cardiac protective effect for dexrazoxane beginning at a later date may have been reduced compared to earlier commencement, there was still evidence of significant protection. The current US Food and Drug Administration approval for dexrazoxane use is restricted to women with breast cancer who have already received 6 cycles of doxorubicin-based chemotherapy.

Dexrazoxane does not appear to alter doxorubicin pharmacokinetics.[74] However, a phase I controlled crossover study examining the pharmacokinetics of epirubicin and dexrazoxane demonstrated an increased systemic clearance of epirubicin, with a resulting decrease in the AUC, in patients receiving higher doses of dexrazoxane.[75] It was reassuring that a randomised controlled trial in women with advanced breast cancer receiving epirubicin-based chemotherapy and dexrazoxane (dexrazoxane to epirubicin dose ratio of 10 : 1) failed to demonstrate any difference in objective response rates, progression-free and overall survival between the 2 arms. The probability of developing chronic cardiac toxicity was significantly lower in the dexrazoxane-treated patients.[70]

Thus, randomised clinical trials have confirmed the chemoprotective action of dexrazoxane against anthracycline-associated cardiac damage, allowing the delivery of higher cumulative doses. This may, in turn, impact upon patient quality of life and control of tumour-related symptoms. There is now also evidence that the delayed introduction of dexrazoxane will reduce the risk of cardiac toxicity. Yet to be confirmed is the issue of whether the use of dexrazoxane in paediatric and young adult patients receiving anthracycline chemotherapy will reduce the risk of late cardiac toxicity. Studies previously described in this section[48,49] have demonstrated the risk of ongoing cardiac toxicity in potential long term cancer survivors who have been treated with anthracyclines. However, it remains unclear whether the early institution of a cardioprotective agent in these patients will reduce this risk, and further investigation is necessary.

4. Mesna

4.1 Mechanism of Action in Oxazaphosphorine-Induced Urotoxicity

The oxazaphosphorine-based alkylating agents, including ifosfamide and cyclophosphamide, undergo metabolic activation by the hepatic microsomal

enzyme system to form phosphoramide mustard and acrolein. Acrolein and other urotoxic metabolites, especially 4-hydroxy metabolites[76] are subsequently excreted intact into the urinary bladder to produce haemorrhagic cystitis.[77] Dependent on the manner in which high dose cyclophosphamide is administered (i.e. bolus infusion or 24-hour continuous infusion) the urinary pharmacokinetics of the alkylating metabolites will vary.[78] When cyclophosphamide is given as a short infusion, urinary concentrations of metabolites peak at 4 to 8 hours. When administered as a 24-hour infusion, alkylating metabolites increase gradually to reach peak concentrations at 18 to 22 hours. In the absence of a chemoprotective agent, ifosfamide and cyclophosphamide are associated with dose-limiting urothelial toxicity.

Mesna (sodium-2-mercapto-ethane sulfonate) has been developed as a specific chemoprotective compound against acrolein-induced bladder toxicity. It is a thiol compound, which inactivates alkylating metabolites by forming an inert thioether. Upon entering the bloodstream, mesna is immediately converted to an inactive disulfide form, dimesna (dithiodiethanesulfate). Dimesna is subsequently filtered and secreted by the kidneys, where the enzymes thiol transferase and glutathione reductase reduce dimesna back to mesna. Mesna is delivered to the bladder, whereupon the free sulfhydryl groups inactivate acrolein.[79] Because the activity of mesna is restricted to the urinary tract, the systemic activity and the non-urological toxicity of the oxazaphosphorines are not affected, and thus it is possible to administer mesna and ifosfamide or cyclophosphamide simultaneously.

4.2 Clinical Trials

Several studies have now confirmed the protective capacity of mesna against ifosfamide and cyclophosphamide-induced bladder toxicity. These have included comparisons with 'standard prophylaxis' including prehydration, urinary alkalinisation and forced urinary diuresis with furosemide[80] or placebo.[81] In the placebo-controlled randomised trial, the incidence of ifosfamide-induced moderate or severe haematuria in intravenous mesna recipients was 6.7% compared with 32% in the placebo group.[81] Chemoprotection with intravenous mesna has also been compared with intravenous hydration and continuous bladder irrigation in patients receiving high dose cyclophosphamide and bone marrow transplant.[82] The overall incidence of haematuria of any grade was significantly higher in the bladder irrigation group (76%) than in the mesna group (53%). However, the incidence of grade III and IV haematuria was equivalent in both groups. There was a statistically significant reduction in the incidence of moderate or severe bladder discomfort ($p < 0.0001$), and incidence of urinary tract infections ($p = 0.03$) in the mesna group.

Clinical trials comparing acetylcysteine with intravenous mesna as a protective agent against ifosfamide-induced urothelial toxicity have confirmed the superiority of mesna. In 1 study, 27.9% of patients receiving acetylcysteine developed haematuria versus 4.2% of mesna recipients.[83] Legha et al.[84] reported a 60% incidence of haematuria in the acetylcysteine group compared with 20% in patients receiving mesna.

4.3 Administration Schedules

Several features of the pharmacokinetics of mesna affect the appropriate scheduling of the drug. The half-life of mesna is only 1 hour,[85] which is far shorter than that of acrolein. Thus, it is necessary to continue treatment with mesna beyond the completion of administration of ifosfamide or cyclophosphamide. Peak urinary thiol accumulation following intravenous mesna is 1 hour, and for the oral route it is 3 hours.[86] The bioavailability of thiols in the urinary bladder is approximately 50% after an intravenous dose, and 35% after an oral mesna dose.[87] Pharmacokinetic studies have demonstrated 40 to 50% bioavailability following oral administration of mesna.[87] To accommodate for the delay in reaching peak urinary thiol levels it is recommended that patients receive a bolus intravenous dose of mesna before chemotherapy. Mesna can then be given intravenously as a continuous infusion during and following chemotherapy,

or as repeat bolus intravenous injections at 4 and 8 hours. A recent randomised study has suggested that equal doses of mesna may be given 15 minutes before and 4 hours after chemotherapy without loss of urothelial protection.[88]

The total intravenous dose of mesna is usually 60% of the oxazaphosphorine dose, although other studies have given the drugs in a 1 : 1 ratio.[82,88] To accommodate the reduced bioavailability of orally administered mesna, doubling of the standard intravenous dose of mesna is recommended in the oral setting. However, it is recommended that oral therapy be reserved only for chemotherapy regimens where emesis is not a major concern, and that the initial dose of mesna should still be administered intravenously.[89]

With the trend towards increased outpatient or ambulatory chemotherapy, and the limitations of intravenous mesna administration, attention has focused on alternative routes of mesna administration, including oral and subcutaneous delivery. A comprehensive review by Goren[90] has analysed the administration schedules and incidence of ifosfamide-induced haematuria in 47 clinical studies, the majority employing a combination approach of initial intravenous followed by oral administration of mesna. He concluded that oral mesna is an attractive alternative to intravenous administration, although the optimal schedule has yet to be established.

Although the experience with continuous subcutaneous infusion of mesna is not as extensive as the oral route, initial studies suggest that it is a practical and effective alternative to intravenous mesna.[91,92]

Mesna is usually associated with minimal toxicity. Mild nausea and vomiting may occur with oral mesna solution, secondary to the sulfur taste, although it appears to be less significant with the tablet preparation. Oral aqueous mesna should be administered diluted in orange juice or carbonate cola. Unusual reported adverse effects of mesna have included allergic contact dermatitis[93] and a hypersensitivity reaction imitating vasculitis.[94]

In summary, mesna is commonly used as a chemoprotective agent in patients receiving ifosfamide and high dose cyclophosphamide chemotherapy. Further studies employing the oral and subcutaneous routes of mesna administration are required to identify the appropriate schedules to optimise outpatient therapy with ifosfamide.

5. Conclusion

There is now substantial evidence in the literature to confirm the role of chemoprotective agents in the management of patients receiving cytotoxic drugs. Dexrazoxane has been officially approved in many countries around the world as a means to reduce anthracycline-induced cardiotoxicity, particularly in women with breast cancer. In addition, mesna is routinely used to attenuate oxazaphosphorine-induced urothelial toxicity and amifostine is being increasingly employed in the prevention of cisplatin-associated neurotoxicity and nephrotoxicity.

All of these agents appear to provide protection without any reduction in the antitumour activity of the simultaneously administered chemotherapy agents, and in certain situations have provided the means to deliver higher cumulative doses of chemotherapy. However, further studies are required to assess whether chemoprotectants will ultimately allow the use of more dose-intensive regimens to overcome tumour resistance and improve patient response rates and survival.

Acknowledgements

Jennifer O'Brien is thanked for her assistance in preparation of this manuscript.

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