Cancer Chemother Pharmacol (1993) 33: 93–106

Cancer Chemotherapy and Pharmacology

Review

WR2721 as a modulator of cisplatin- and carboplatin-induced side effects in comparison with other chemoprotective agents: a molecular approach

Marco Treskes, Wim J. F. van der Vijgh

Department of Oncology, Free University Hospital, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

Received 11 April 1993/Accepted 13 May 1993

Abstract. Cisplatin is an active cytostatic that became successful in the treatment of several types of solid tumours after its nephrotoxic potential was controlled by hydration and diuresis. Thiol compounds were tested to reduce further cisplatin-induced nephrotoxicity. Thiosulphate is rapidly excreted by the kidneys and protects against cisplatin-induced nephrotoxicity by inactivating reactive platinum species in the kidney. Due to inactivation of cisplatin in the circulation, thiosulphate also interferes with its antitumour activity. Therefore, it is mainly used in two-route schedules, whereby cisplatin is delivered locally to the tumour (i.p. or i.a.) while systemic (i.v.) thiosulphate protects the kidneys. Diethyldithiocarbamate was shown to protect against cisplatin-induced nephrotoxicity in several animal models by reversing cellular damage. However, in the clinic it has been less successful, partly due to its central nervous system toxicity. The endogenous thiol compounds glutathione and metallothionein have been shown to reduce cisplatin-induced toxicity both in animal models and in clinical trials. However, the results are rather preliminary and a reduction in therapeutic efficacy may be expected, for both glutathione and metallothionein have been reported to be involved in platinum resistance. The thioether methionine has been shown to reduce cisplatin-induced nephrotoxicity in animal models but it has not yet been tested in the clinic. Cisplatin-induced acute emesis can be sufficiently controlled with a new class of 5-hydroxytryptamine-3 ($5HT_3$)-receptor blockers, but delayed emesis remains a problem. High-dose cisplatin regimens with protection of the kidneys induces ototoxicity, peripheral neuropathy and myelotoxicity, which become dose-limiting. Neurotoxicity was partly reversed by the neurogenerative agent ORG2766, but this agent does not reduce other cisplatin-induced toxicities. Therefore, an agent capable of protecting multiple non-tumour tissues is needed. Carboplatin is a second-generation analogue of cisplatin with less nephro-, neuro- and ototoxicity. Carboplatin is at least as active as cisplatin at its maximum tolerated dose, which is defined by its myelotoxicity. Protection from carboplatin-induced myelotoxicity may be controlled by autologous bone marrow transplantation and/or hematopoietic growth factor infusions. High-dose carboplatin schedules may cause nephrotoxicity, neurotoxicity and ototoxicity. Again, the protection of multiple non-tumour tissues is needed. WR2721 appears to be such a modulating agent capable of protecting multiple non-tumour tissues. It was shown to be preferentially metabolized and taken up as the thiol metabolite WR1065 by non-tumour tissues as compared with (hypoxic) solid tumours. It was shown to protect mice from cisplatin-induced nephrotoxicity and from cisplatin- and carboplatin-induced myelotoxicity without interfering with the antitumour activity. The first clinical studies suggest the same selective protection of multiple non-tumour tissues from cisplatin-induced toxicity. This could be explained by a strong prevention (not reversal) of cisplatin-induced cellular damage by WR1065, whereas WR2721 or its main metabolites will hardly inactivate the intact platinum-based drug in the circulation.

Introduction

The treatment of cancer plays an important role in modern medical science. Non-operable and/or metastasized tumours may be fought with DNA-damaging agents (radiation and/or cytostatic drugs) to prevent tumour cell proliferation. An important group of cytostatic drugs in the chemotherapeutic treatment of several types of solid tumour are the platinum-based antitumour drugs.

After the somewhat serendipitous discovery of the cytostatic properties of cisplatin [*cis*-diamminedichloroplatinum(II), Fig. 1] by Rosenberg et al. [137], it was almost discarded as an antitumour agent because of its nephrotoxicity [185]. Following the reduction of this toxicity by

Offprint requests to: M. Treskes

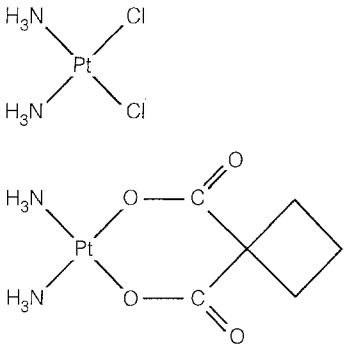

Fig. 1. Structural formulae of cisplatin (*top*) and carboplatin (*bottom*)

forced hydration, cisplatin became one of the most successful agents in cancer chemotherapy, showing activity against several solid tumours [111].

Over 2,000 analogues of cisplatin have been synthesized and tested as second-generation antitumour drugs to obtain Pt complexes with reduced toxicities and similar or improved antitumour activity. At the moment, carboplatin [diammine(1,1-cyclobutane-dicarboxylato)platinum(II); Fig. 1] is one of the few successful analogues with clinical application. Its dose-limiting toxicity is bone marrow suppression [186].

Mechanisms of antitumour activity

It is generally accepted that the antitumour activity of the platinum compounds is the result of their binding to DNA [133, 154]. This binding is preceded by the hydrolysis of the labile anionic ligand(s). The hydrolysis of the monofunctional chloro ligand in cisplatin is 100 times faster than that of the dicarboxylato ligand in carboplatin [89]. However, because carboplatin is given at a higher dose and because it is less rapidly cleared from the plasma than cisplatin [181], the tumour is exposed for a longer time and to higher levels of drug after carboplatin administration than after treatment with cisplatin. Ultimately, carboplatin and cisplatin form the same adducts with DNA; only the reaction kinetics differ [89]. The binding of the *cis*-diammine platinum(II) moiety with two adjacent guanosines on the same DNA strand (PtGG) is the most abundant lesion (ca. 62%), followed by the *cis*-diammine platinum(II) moiety bound to a guanosine and an adjacent adenine on the 5' side of the same strand (PtAG, ca. 18%), the *cis*-diammine platinum(II) moiety bound to two guanosines on the same strand separated by one base or bound to two guanosines on opposite DNA strands (GPtG, ca. 8%) and the *cis*-diammmine platinum(II) moiety monofunctionally bound to a guanosine at the moment the reaction is stopped with ammonium bicarbonate (PtG, ca. 8%). Surprisingly, binding of the *cis*-diammine platinum(II) moiety to guanosine and an adenosine on the 3' side on the same strand (PtGA) has not been observed. From alkaline elution studies (to quantitate interstrand cross-links) it has been concluded that the GPtG mainly originated from *cis*-diammine platinum(II) bound to two non-adjacent guanosines on the same strand [51].

Which Pt-DNA adduct is to be held responsible for the antitumour activity of cisplatin and carboplatin remains a matter of debate. The observation that the trans-analogue of cisplatin, which is incapable of forming intrastrand cross-links with neighbouring nucleotides on the DNA, is hardly antitumour-active suggests that one or more of these intrastrand cross-links should be responsible for the antitumour effect [26, 133, 154]. Elegant analytical methods using liquid chromatography and off-line flameless atomic absorption spectroscopy (FAAS) or a competitive enzyme-linked immunosorbent assay have enabled the identification and quantification of the various Pt-DNA adducts at the femtomolar level [47, 51, 52, 53, 131]. Immunohistochemical techniques have allowed the quantification of Pt-DNA adduct levels in cultured cancer cells and in (tumour) tissue samples from animals and patients treated with cisplatin or carboplatin [38, 53, 166–169]. At present, most authors suggest a relationship between the antitumour activity and the intrastrand cross-links of cisplatin (and carboplatin) to the N_7 position of two adjacent guanosines. This Pt-GG adduct is the most abundant lesion and is thought to introduce a distortion in the DNA that is large enough to stop the division of the cells without being rapidly recognized and, thus, efficiently removed by repair enzymes [26]. Recent results obtained in preclinical studies demonstrated a relationship between the Pt-DNA adduct levels in cultured tumour cells and the cytostatic effect [125, 168]. However, a cisplatin-resistant IgM immunocytoma did not differ in the formation and/or repair of Pt-DNA adducts from the cisplatin-sensitive parental tumour [52]. This finding may be explained by the recent discovery that contrary to the data on total Pt-DNA levels, low levels of Pt-DNA adducts in actively transcribed regions of specific genes correlate with resistance [194]. However, a tentative relationship has been demonstrated between Pt-DNA adduct levels and antitumour response in cultured cells [168, 169] and in white blood cells of platinum-treated patients [126, 132].

Recently, new possible cellular targets involved in signal transduction pathways have been reported to be involved in the cytostatic actions of platinum antitumour agents [11]. Nevertheless, at present DNA is considered to be the major cellular target involved in the cytotoxic actions of platinum-based antitumour agents. Therefore, the study of these adducts remains important in the elucidation of the clinical effects of cytostatic platinum compounds.

Cisplatin- and carboplatin-induced toxicities and their modulation

Platinum antitumour agents show a steep dose-response curve for their antitumour activity in several tumour types. This means that a decrease in the dose-limiting toxicity allows for the safe administration of a higher dose, thus improving the therapeutic efficacy [122]. The reduction of platinum-induced toxicities by modulating agents may therefore be a realistic approach to improve the therapeutic effect.

Cisplatin-induced nephrotoxicity

The use of cisplatin in the clinic was originally hampered by its nephrotoxicity [185]. Methods of overcoming this toxicity have included a variety of techniques, among which were the development of second-generation platinum compounds with reduced nephrotoxic potential and the use of modulating agents that could selectively reduce the cisplatin-induced nephrotoxicity. Therefore, the mechanisms underlying this toxicity have also become the subject of extensive research. An excellent review on cisplatin-induced nephrotoxicity has been published elsewhere [35].

It has been shown that Pt is accumulated in the kidney and that tubular damage is the main cause of cisplatin-induced nephrotoxicity. Apart from glomerular filtration, active transport of low-molecular weight metabolites takes place to and from the lumen of the kidney tubules [140]. Low-molecular-weight metabolites of cisplatin may be reactive and, thus, involved in the effects (toxicities) of cisplatin. Because of the many analytical chemical difficulties encountered in the selective and sensitive detection of these metabolites, our knowledge about their identity and effects is very limited [33, 107]. Cisplatin bound to plasma proteins has lost most of its antitumour and toxic activity [161]. Cisplatin-methionine interaction products have been identified and do not appear to play a role in cisplatin-induced nephrotoxicity, in contrast to the reactive hydrolysis species for which it is clear that they play a role in cisplatin-induced nephrotoxicity [33].

The precise cellular target of cisplatin-induced nephrotoxicity remains a matter of debate. As possible causes of cisplatin-induced nephrotoxicity, the following have been reported: depletion of cellular thiols [190]; inhibition of the synthetic ability of the cell by damage to the DNA [164]; inhibition of post-transcriptional protein synthesis [108]; interference with Ca^{2+} homeostasis [40]; inhibition of adenosine triphosphatases (ATPases) [117], which has been contradicted by other investigators [177]; and damage to mitochondria [27, 56, 63]. Increased lipid peroxidation has also been reported as a cause of cisplatin-induced nephrotoxicity by some authors [20, 22, 152], but not by others [8]. The involvement of radicals may be secondary to cisplatin-induced damage to defence systems of the cell such as the glutathione-mediated detoxification of radicals [22].

Modulation of cisplatin-induced nephrotoxicity

Apart from the development of cisplatin analogues, the development of modulating agents to reduce selectively the nephrotoxic potential of cisplatin has received much attention. The reduction of high peak plasma levels of cisplatin by prolonged infusion, the reduction of the residence time of reactive Pt species in the kidney by diuresis and the inhibition of hydrolysis by (hypertonic) saline infusion have dramatically decreased cisplatin-induced nephrotoxicity [23, 54]. Agents modulating renal blood flow [39, 70, 150] and agents modulating the cellular uptake of Pt species [16, 32, 34, 45, 80, 81, 127, 150] have produced contradictory results.

Radical scavengers [29, 43, 195] have been shown to reduce cisplatin-induced nephrotoxicity, although the role of radicals in cisplatin-induced nephrotoxicity remains a matter of debate [8, 20]. Several other modulating agents with unknown mechanisms of protection have also been reported to modulate cisplatin-induced nephrotoxicity [44, 93, 121, 156].

Besides diuresis and hydration, the use of nucleophilic thiols or thioethers with a high affinity for the electrophilic Pt(II) species appears to be a successful approach to modulate cisplatin-induced nephrotoxicity [23]. The following modulators have been used or are presently under (clinical) investigation.

Thiosulphate has been shown to be reactive towards Pt(II) [36, 48] and to concentrate in the kidneys [148]. Therefore, thiosulphate reduces cisplatin-induced nephrotoxicity by inactivating toxic Pt species in the kidney, and this allows the use of an increased dose of cisplatin [96]. Because thiosulphate has also been shown to inactivate cisplatin in the blood [3, 79, 88], exposure of the tumour to active Pt species is also reduced [60, 61]. Indeed, thiosulphate has been shown to reduce the antitumour activity of cisplatin in tumour cell cultures [31, 179]. Concomitant i.v. administration of thiosulphate with high-dose cisplatin has failed to increase the therapeutic index (the antitumour efficacy at a certain level of toxicity) in rodent models [1, 72, 76]. However, an increased plasma level of dethyldithiocarbamate (DDTC)-reactive platinum has been observed in patients treated with a higher, equitoxic dose (the amount of cisplatin given together with a protective agent that establishes a level of toxicity similar to that produced by cisplatin alone) of cisplatin plus thiosulfate as compared with cisplatin alone [61]. This finding corresponds to the reduced nephrotoxicity observed by other investigators in the clinic [71, 102] without an apparent loss of antitumour effect. However, this regimen was associated with high neurotoxicity.

Thiosulphate may be particularly useful in two-route regimens, i.e. the tumour is locally exposed to cisplatin (i.p. or i.a.) while systemic (i.v.) thiosulphate is preferentially delivered to the kidney. Cisplatin given i.p. in combination with i.v. thiosulphate has resulted in an increased therapeutic index in a mouse tumour model [78]. In the clinic, i.p. cisplatin plus i.v. thiosulphate has been shown to be associated with reduced toxicity [4, 73, 74, 95, 100, 162]. The use of high-dose cisplatin plus etoposide (i.p.) with i.v. thiosulphate protection has produced high response rates in ovarian cancer patients, but it is too early for this regimen to be reliably compared with other platinum-based therapies with regard to antitumour activity [75]. An increased exposure to reactive Pt has been observed for the peritoneal cavity [4, 73, 74]. Local (i.a.) cisplatin with systemic (i.v.) thiosulphate has been shown to be very active in several rodent tumours of the limb, liver and uterus, especially when i.a. cisplatin is combined with angiotensin II [68, 69, 90, 91, 94, 178]. When this approach is used for the treatment of liver tumours in the clinic, the higher equitoxic dose of cisplatin results in elevated Pt levels in plasma, although the nephrotoxicity is

reduced [2, 160, 163]. An evaluation of the antitumour activity has not yet been possible.

An interesting, recent finding is the reduction of cisplatin-induced chromosomal damage (sister-chromatid exchanges) by thiosulphate given concomitantly with or 3 h after cisplatin [118]. The administration of thiosulphate 3 h after cisplatin might thus reduce genotoxic effects that may result in the occurrence of secondary tumours [85] without decreasing the antitumour efficacy. However, thiosulphate given on this schedule will have lost its potency as a modulator of cisplatin-induced nephrotoxicity. It can be concluded that despite extensive research, a clear increase in the therapeutic index has not yet been achieved for cisplatin given in combination with thiosulphate, and this regimen is therefore not generally used in the treatment of cancer.

DDTC is very reactive towards Pt(II) complexes [36]. When given 1–4 h after cisplatin, it has been shown to rescue kidneys from cisplatin-induced damage without producing a reduction in the antitumour activity [18, 24, 25]. This protection has also been observed in vitro with cultured porcine kidney tubule cells [108]. It was hypothesized that DDTC could reverse the formation of cisplatin-protein complexes, which are responsible for (part of the) cisplatin-induced nephrotoxicity, but not that of cisplatin-DNA complexes. This hypothesis was supported by the in vitro restoration of protein function by DDTC after inactivation with cisplatin, whereby DDTC failed to reverse cisplatin-DNA binding [17, 62]. DDTC was shown to reverse Pt-methionine binding quickly, but not Pt-cysteine binding, thus suggesting that the rescue of cisplatin-induced nephrotoxicity by DDTC results from reversal of the formation of Pt-methionine and not Pt-cysteine complexes [97]. Surprisingly, thiosulphate could also rapidly reverse the Pt-methionine bond, but in contrast to DDTC, it was not capable of reactivating cisplatin-inactivated fumarase. This effect might have been due to the negative charge and hydrophilicity of thiosulphate, preventing its access to the platinated methionine residue in the active centre of the enzyme [19].

When applied after cisplatin in several rodent models, DDTC and various other dithiocarbamates have resulted in an increase in the excretion of Pt in bile and urine [12, 87, 92] and a decrease in Pt levels in normal tissues [12, 87, 135], which might explain the observed reduction in nephrotoxicity. Rescue from cisplatin-induced nephrotoxicity by dithiocarbamates was increased when DDTC was given both 24 h prior to cisplatin to induce metallothionein expression and daily × 6 after cisplatin to reverse cisplatin-induced damage [82]. However, the induction of metallothionein in the tumour might result in a reduced sensitivity to cisplatin, as metallothionein has been shown to be involved in cisplatin resistance [7, 129, 139, 142]. Cisplatin and local hyperthermia used in combination with various dithiocarbamates has been very effective in several rodent tumour models [113, 187]. In clinical trials, DDTC given 45 min after cisplatin did not change (filterable) Pt pharmacokinetics [37, 124]. Protection against cisplatin-induced nephrotoxicity has been observed by some investigators [15, 130] but not by others [124]. The use of DDTC in the clinic is hampered by a severe but reversible toxicity to the central nervous system, requiring heavy sedation of the patient [15, 130, 138]. *Disulfiram*, the orally available dimer of DDTC, did not reduce cisplatin-induced nephrotoxicity in a phase II trial [182].

Mercaptoethanesulphonate (mesna) has also been tested for its protective potential against cisplatin-induced nephrotoxicity. Early animal studies showed some protection [86], but later studies failed to demonstrate any protection [105]. The low reactivity of mesna with toxic Pt species as compared with thiosulphate may be an explanation for this lack of protection [96].

Methionine, a sulphur-containing amino acid, has been shown to react with cisplatin [33]. Although Pt-methionine complexes can be reversed by strong nucleophiles, the interaction products of cisplatin with methionine are expected to be less reactive and, thus, less (nephro)toxic. This effect has been confirmed in two separate studies in rodents [33, 120]. Although there is one report of methionine enhancing cisplatin-induced nephrotoxicity in rats [191], recent and detailed animal studies have again shown a protective effect for methionine and other related thioethers [13, 14, 83].

Glutathione (GSH), a cysteine-containing tripeptide, is present in cells in the millimolar range and plays a major role in detoxification processes [106]. Because of its nucleophilic thiol moiety [36], it is expected to play a role in the intracellular inactivation of cytotoxic Pt species. There is one report on the reduction of cisplatin-induced nephrotoxicity by depletion of GSH in rats [103], which is contradicted by other studies demonstrating increased nephrotoxicity upon GSH depletion [77, 109]. Furthermore, GSH has been demonstrated to decrease cisplatin-induced nephrotoxicity in animals [6, 155, 196] and in patients [42]. Although there are several reports on the involvement of GSH in the resistance of tumour cells to cisplatin [7, 129, 139, 142], an apparent reduction in the antitumour activity of cisplatin by GSH has not been observed [42, 196].

Conflicting results have been reported on the induction of renal *metallothioneins* (MT), cysteine-rich low-molecular-weight peptides, by cisplatin [50, 109, 134, 155]. Protection against cisplatin-induced nephrotoxicity has been demonstrated by the induction of MT with metals such as Bi and Zn [20, 66, 115, 116]. Other investigators have observed a slightly protective effect [151, 155] or no protection at all [109, 134]. An increased binding of Pt was not observed in the MT fractions of kidney and liver cells [20, 145, 155]. Radical scavenging by MT has been proposed as a possible mechanism of its protection against cisplatin-induced nephrotoxicity [20]. Although MT appears to play a role in the resistance of some tumour cell lines to cisplatin [7, 129, 139, 142], no reduction in the antitumour activity has thus far been observed [116].

Selenite and ebselen are compounds containing *selenium*, which is a heavier member of the same group as sulphur in the periodic table of the elements. These compounds have protected mice and rats from cisplatin-induced nephrotoxicity without producing a reduction in the antitumour activity [9, 10, 114, 119, 141, 153, 188]. It is suggested that selenium compounds may protect the kidneys from damage by free radicals [153]. However, the involvement of radicals in cisplatin-induced nephrotoxic-

ity is very uncertain [8, 20]. In analogy to sulphur compounds, it can be postulated that selenium compounds act simply by inactivating reactive Pt species via ligand exchange. This possibility has not yet been studied, and more research on the molecular basis of the protective action of selenium compounds has to be performed.

In conclusion, thiosulphate can reduce cisplatin-induced nephrotoxicity but has a major drawback in that it inactivates cisplatin in the blood. Even in two-route chemotherapy regimens, its applicability has not yet been proven. DDTC is capable of reversing cisplatin-induced damage in the kidney. However, its severe neurotoxicity makes DDTC very unsuitable for clinical application. Methionine and related compounds such as GSH and the induction of metallothioneins can reduce cisplatin-induced toxicity. However, these remain in an early stage of development and problems involving a reduction in the antitumour efficacy may be expected.

Cisplatin- and carboplatin-induced bone marrow suppression

With cisplatin, bone marrow suppression occurs only in high-dose regimens [122]. For carboplatin, myelotoxicity is the dose-limiting toxicity [186]. The molecular mechanism behind the myelotoxic action of cisplatin and carboplatin has not been studied. It is very likely that the division of the rapidly proliferating myeloid progenitors is inhibited by Pt-DNA adduct formation. This implies that the selective modulation of bone marrow suppression is probably not possible on a pharmacodynamic basis as the tumour and the bone marrow share the same mechanism (cellular target) of cytotoxicity.

Modulation of cisplatin- and carboplatin-induced bone marrow suppression

The modulator of cisplatin-induced nephrotoxicity, DDTC, also protects mice from cisplatin-induced bone marrow suppression when given after cisplatin [18, 41, 49, 65, 123]. Myelosuppression caused by carboplatin has also been reduced by this modulator [41, 65, 143]. However, the dependence of timing was less obvious for this effect than for modulation of cisplatin-induced nephrotoxicity, and there was no clear dose-response relationship [65, 123]. Dible et al. [41] observed maximal protection when DDTC was administered concomitantly with cisplatin, which also resulted in a reduction in the antitumour activity. In a recent report, it was demonstrated that a DDTC-mediated release of growth factors by stromal cells occurred instead of a direct reversal of damage to the colony-forming units (CFUs, early myeloid progenitors) [144]. This means that the Pt-induced damage was somewhat compensated and not reversed. In clinical trials of cisplatin with dithiocarbamate rescue, an inhibition of myelotoxicity has not yet been demonstrated. In a clinical trial of carboplatin with DDTC, severe myelotoxicity was encountered that might have been related to previous treatments. Even when this was the case, the protection provided by DDTC could not have been impressive [138]. Mesna did not reduce bone marrow suppression in cisplatin-treated mice [98].

Thus, DDTC and mesna do not hold a lot of promise for protection against platinum-induced myelotoxicity. To date, laborious autologous bone marrow transplantation and/or the use of peripheral stem cell infusions and haemopoietic growth factors have been the only possibilities of circumventing the myelotoxic effects of platinum cytostatics [110, 149, 165]. However, recombinant erythropoietin did not protect murine erythroid stem cells from cisplatin-induced cytotoxicity in vitro [55]. Thus, bone marrow remains one of the non-tumour tissues that are affected by platinum toxicity and may limit the dose that can be applied safely.

Cisplatin- and carboplatin-induced neurotoxicity and ototoxicity

Cisplatin causes a loss of mainly sensory nerve function that appears to be related to the cumulative dose [21, 136, 170]. Thus far, no study has been performed on the molecular mechanisms of this side effect. The cellular target is not known. Morphology studies have revealed changes in DNA, the nucleolus and the number of lysosymes [112]. High-dose cisplatin is associated with ototoxicity, which appears to be reduced when infusion rates are reduced [184]. Carboplatin is less ototoxic than cisplatin. However, high-dose carboplatin with autologous bone marrow support has been associated with substantial ototoxicity [149].

Modulation of cisplatin-induced neurotoxicity and ototoxicity

Neurotoxicity has been studied in clinical trials with modulating agents that were initially developed for the reduction of nephrotoxicity. Thiosulphate has offered protection against cisplatin-induced neurotoxicity [101]. However, a recent phase I trial with high-dose cisplatin and thiosulphate (i.v.) demonstrated a dose-limiting neurotoxicity [102]. For DDTC, no protection against cisplatin-induced neurotoxicity has yet been demonstrated and no protection against cisplatin-induced ototoxicity has been observed [15, 124]. Quite to the contrary, the orally available dimer of DDTC, disulfiram, has been shown to reduce cisplatin-induced ototoxicity (but not nephrotoxicity) [182]. The neurotropic peptide and adrenocorticotropic hormone ($ACTH_{4-9}$) analogue ORG.2766, involved in nerve regeneration, has ameliorated cisplatin-induced neurotoxicity both in animal studies and in the clinic [57, 58, 112].

In conclusion, cisplatin-induced neurotoxicity (and ototoxicity) is the main problem associated with high-dose cisplatin treatment regimens. Although some success has been obtained with the nerve regeneration-stimulating agent ORG.2766, true protection (prevention of damage) has not yet been established.

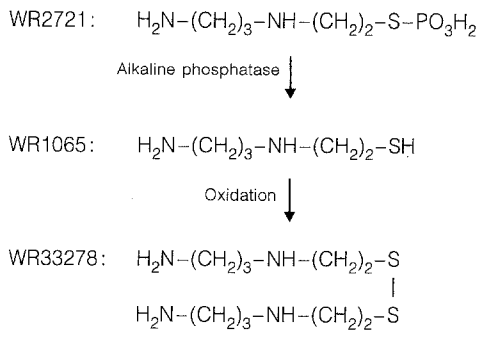

Fig. 2. Structural formula of WR2721 and its conversion into the main metabolites WR1065 and WR33278

Gastro-intestinal toxicity

Cisplatin and, to a lesser extent, carboplatin cause severe but reversible gastro-intestinal toxicity, resulting in nausea and vomiting and diarrhoea. Especially the nausea and vomiting caused by platinum compounds is very severe and lays a heavy burden upon the patient [183].

Modulation of gastro-intestinal toxicity

Conventional antiemetics reduce cisplatin-induced nausea and vomiting, although severe, to an acceptable level [183]. With the development of a new class of antiemetic agents [serotonin (5-hydroxytryptamine-3, $5HT_3$)-receptor antagonists], the occurrence and severity of acute nausea and vomiting has been dramatically reduced [128]. This effect is very important in preventing treatment refusal.

Cisplatin-induced toxicity to the intestine, resulting in diarrhoea, is transient and does not produce problems in the clinic [183]. Therefore, the mechanism and modulation of this toxicity has not received much attention. Dithiocarbamates have been shown to reduce histopathological damage to the small intestines of cisplatin-treated rats [92] and to be antiemetic in dogs [18]. Disulfiram, the dimer of DDTC, has reduced nausea and vomiting in a phase II trial [182]. The uroprotector mesna protects cisplatin-treated mice from gastro-intestinal toxicity and lethality [5]. Bi^{3+}-induced metallothioneins have reduced gastro-intestinal toxicity in cisplatin-treated mice [116].

In conclusion, cisplatin-induced nausea and vomiting are, although not life-threatening, major problems from a patient's point of view. At present, acute nausea and vomiting can be satisfactorily controlled, but delayed vomiting remains a problem [128]. Intestinal toxicity causing diarrhoea has not been a major problem and its modulation has not been studied much.

We can conclude that the reduction of cisplatin-induced nephrotoxicity by (saline) hydration and diuresis has enabled the safe use of cisplatin at a dose at which especially ototoxicity and neurotoxicity become a problem. A new generation of antiemetics ($5HT_3$-receptor blockers) has considerably improved the tolerance of patients to platinum chemotherapy. Carboplatin is an active cisplatin analogue with low nephrotoxic potential; the dose-limiting toxicity of this compound is myelotoxicity. However, when given with bone marrow protection, high-dose carboplatin may also cause nephrotoxicity and ototoxicity [67, 149]. Therefore, a modulator that protects selectively against the multiple side effects (nephro-, myelo-, neuro- and ototoxicity) of platinum antitumour drugs is needed. At present, the most promising compound for the achievement of this goal is WR2721.

WR2721

WR2721 [S-2-(3-aminopropylamino)ethylphosphorothioic acid; ethiophos; Fig. 2] was developed by the Walter Reed Army Institute Hospital to protect soldiers from radiation on the battle field. WR2721 is the less toxic pro-drug of the aminothiol compound WR1065. WR1065 is rapidly formed in vivo by dephosphorylation of WR2721 [28, 147]. Aminothiol compounds are well known for their radioprotective potential. The mechanisms underlying this protection are the depletion of oxygen, reducing the formation of reactive oxygen radicals, the inactivation of radicals by a direct interaction and proton donation to DNA damaged by radicals [28]. It was an obvious step to the use of the pro-drug WR2721 as a protector in radiotherapy.

WR2721 has been shown to protect selectively non-tumour tissues against radiation-induced damage [28]. This selectivity could be explained by the preferential accumulation of WR1065 in non-tumour tissues as observed in several species [147]. The preferential accumulation of WR1065 in non-tumour tissues might be explained as follows. WR2721 is dephosphorylated into the protecting thiol metabolite WR1065 by alkaline phosphatase, abundantly present at the cellular membrane of endothelial cells of small blood vessels. Subsequently WR1065 is capable of passively crossing the cellular membrane [30]. Because solid tumours eligible for radiotherapy are poorly vascularized, their alkaline phosphatase activity is low. Furthermore, because of the hypoxic state of the tumour, the tissue pH is decreased. This results in a reduced formation and uptake of WR1065 in the tumour as compared with other tissues via (a) a decreased activity of the alkaline phosphatase present and (b) an increased amount of protonated amino groups in WR1065, decreasing the fraction of neutral molecules that can enter the cell [28, 30, 147]. Thus, selectivity is achieved by the preferential formation and uptake of WR1065 in non-tumour tissues.

Pharmacokinetics of WR2721 and its metabolites

The (tissue) kinetics of WR2721 and its main metabolites have been studied in several species. In all species investigated (including humans), the half-life of WR2721 in the plasma was short (<5 min) [147, 157]. During a 15-min continuous infusion of 740 mg/m^2, the steady-state plasma level of WR2721 was 0.1 m*M* [147]. WR1065 plasma levels were not determined in this study. When 750 mg/m^2 WR2721 was given to a patient in five repeated (150 mg/m^2) bolus injections over 15 min, a steady-state plasma

Table 1. The direct interaction of cisplatin and carboplatin, the prevention and reversal of cisplatin-DNA interactions and the reversal of cisplatin-protein interactions with WR2721 and its main metabolites WR1065 and WR33278 in comparison with DDTC and TS

Modulating agent	$k_{2,CDDP} \times 10^4$ (M^{-1} s^{-1})[a]	$k_{2,CBDCA} \times 10^4$ (M^{-1} s^{-1})[b]	CDDP-DNA Prevention (%)[c]	CDDP-DNA Reversal (%)[d]	Pt(dien)SMe Reversal ($t_{1/2}$, min)[e]	Fumarase Reactivation (%)[f]
WR2721	25.3	6.07	51	14	2187	7.7
WR1065	49.1	12.4	74	28	81.1	16
WR33278	8.60	0.39	64	13	>>	0
DDTC	–	–	97	43	3.15	61
TS	577	82.4	89	13	1.15	–

[a, b] Second-order rate constant for cisplatin (CDDP) and carboplatin (CBDCA) in 10 m*M* phosphate buffer (pH 7.4) at 37° C (after [171])
[c] Prevention of DNA platination by 25 μg cisplatin/ml in the presence of a 50-fold molar excess of modulating agent; incubated for 1 h at pH 7.0 and 37° C (after [172])
[d] Reversal of DNA platination by a 50-fold molar excess of modulating agent; incubated for 2 h at pH 7.0 and 37° C (after [172])
[e] Reversal of the platinum-methionine type binding in Pt(dien) bound to S-methyl-glutathione in 10 m*M* phosphate (pH 7.4) at 37° C (after [173])
[f] Restoration of fumarase activity by 20 m*M* of WR compound or 2 m*M* DDTC after inactivation of the enzyme with cisplatin; incubations were performed in 0.1 *M* phosphate (pH 7.4) at 37° C (after [173])

level of 1 m*M* WR2721 was achieved. The concentration of WR1065 in the plasma of this patient was initially 100 μ*M* and declined slowly to 35 μ*M* after 1 h [146]. WR1065 given i.v. to dogs and monkeys was rapidly distributed from the plasma to the tissues. The terminal half-life was long, probably due to its release from a pool of mixed disulphides [99, 104, 158, 159]. In mice injected with WR2721, WR1065 is rapidly and preferentially taken up into various tissues such as the kidney and lung. These non-tumour tissues accumulate high levels of WR1065 as compared with the tumour, with a subsequent rapid decrease being observed ($t_{1/2}$ <10 min) [147, 180]. The tissue kinetics of WR1065 in bone marrow have not yet been established, but radioprotection studies indicate that WR1065 rapidly enters the bone marrow [28].

It can be concluded that after the i.v. administration of WR2721, its thiol metabolite WR1065 is rapidly and preferentially accumulated by non-tumour tissues. This pharmacokinetic selectivity provides a basis for the selective modulation of platinum-induced toxicities.

WR2721 as a modulator of toxic side effects of platinum antitumour agents

The above-mentioned characteristics of WR2721 make it a promising candidate for the selective modulation of platinum-induced toxicities. Indeed, a decrease in cisplatin-induced nephrotoxicity has been obtained using 100–200 mg/kg WR2721 (dose-modification factors of about 1.5) without producing a reduction of the antitumour activity in mice and rats [84, 192, 193]. In mice, bone marrow suppression by cisplatin has also been decreased by the administration of 600 mg/kg WR2721 (dose modification factor of 3) without resulting in a reduction of the antitumour activity [189]. A very recent study demonstrated the protection of colony-forming bone marrow cells by WR2721 after an in vitro exposure to carboplatin, whereas three human glioblastoma cell lines were not protected [46]. Preliminary results obtained with the combination of carboplatin and WR2721 in tumour-bearing mice have also suggested a selective protection against myelotoxicity [64].

Because of prior investigations of WR2721 as a radioprotector in humans, it was readily available for safe use in clinical trials. WR2721 administration is limited by emesis and reversible hypotension, the latter becoming more severe with increasing infusion time. A maximum tolerated dose (MTD) of 740–910 mg/m^2 has been reached in clinical trials [59]. Clinical trials of cisplatin in combination with WR2721 have shown a decrease not only in myelotoxicity and nephrotoxicity but also in neurotoxicity and ototoxicity without an apparent reduction of the antitumour effect [59].

Recently, mechanistic in vitro studies have been performed to obtain a better understanding of the molecular mechanisms behind the protective action of WR2721 in platinum-based chemotherapy. Cisplatin and its second-generation analogue carboplatin did not react rapidly with the modulating agent WR2721, its thiol metabolite WR1065 or the symmetrical disulphide WR33278 [171] (Table 1). Therefore, it has been postulated that cisplatin or carboplatin will not be significantly inactivated in the circulation by WR2721 or its metabolites, even when they are given at the same time. A dramatic reduction of the formation of cisplatin-DNA adducts was observed in the presence of the WR compounds [172] (Table 1).

WR1065 was the most active compound, its activity being almost comparable with that of the strong nucleophiles DDTC and thiosulphate (TS). This effect has been explained by the ability of WR1065, in contrast to TS and DDTC, to concentrate near DNA by counter-ion condensation, thus inactivating cisplatin and hydrolysed species before they can bind to DNA. Reversal of the formation of cisplatin-DNA adducts also occurred with WR1065 and, to a lesser extent, WR33278 and WR2721. WR1065 was even more active than TS and almost as active as DDTC. However, adduct reversal was slow as compared with the prevention of their formation [172] (Table 1). The reversal of platinum-DNA interactions by thiol compounds contrasts with earlier findings [17, 62]. This effect is prob-

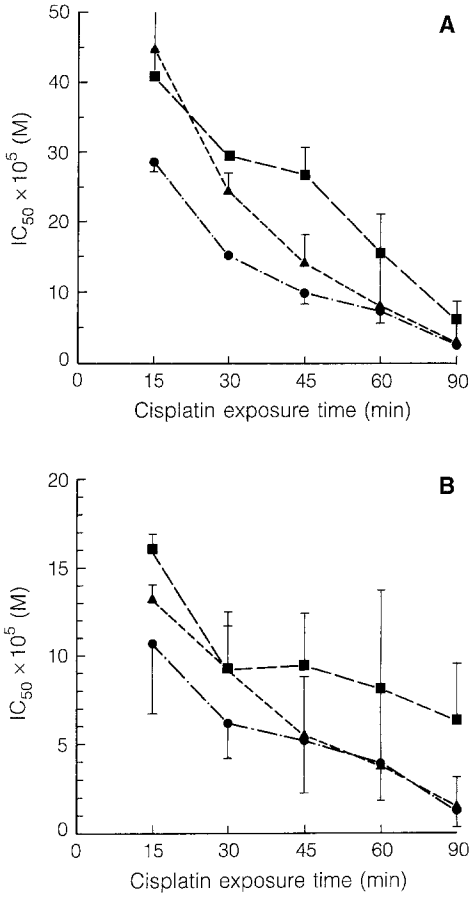

Fig. 3 A, B. IC_{50} values of cisplatin (mean ± SD) for OVCAR-3 cells after various periods of incubation with cisplatin alone (●), with cisplatin after a 15-min incubation with 5 m*M* WR1065 (▲) and after concomitant incubation with cisplatin and 5 m*M* WR1065 (■). Two separate experiments (**A, B**) were performed in triplicate (after [174])

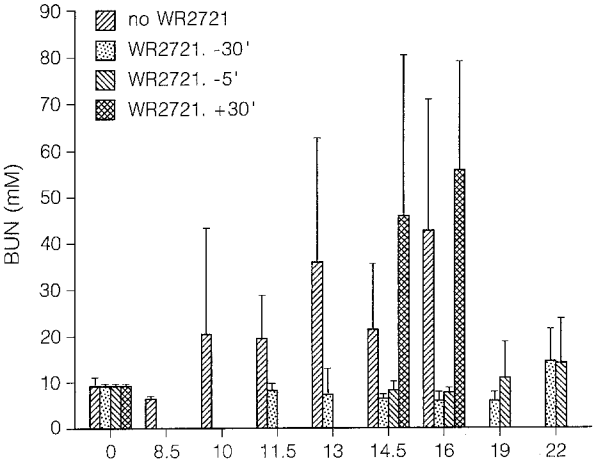

Fig. 4. Modulation of cisplatin-induced nephrotoxicity in BALB/c mice ($n = 8$) by WR2721 (200 mg/kg, i.p.) at 30 min before, 5 min before or 30 min after cisplatin administration as measured by plasma urea levels (m*M*) at day 4 (after [175])

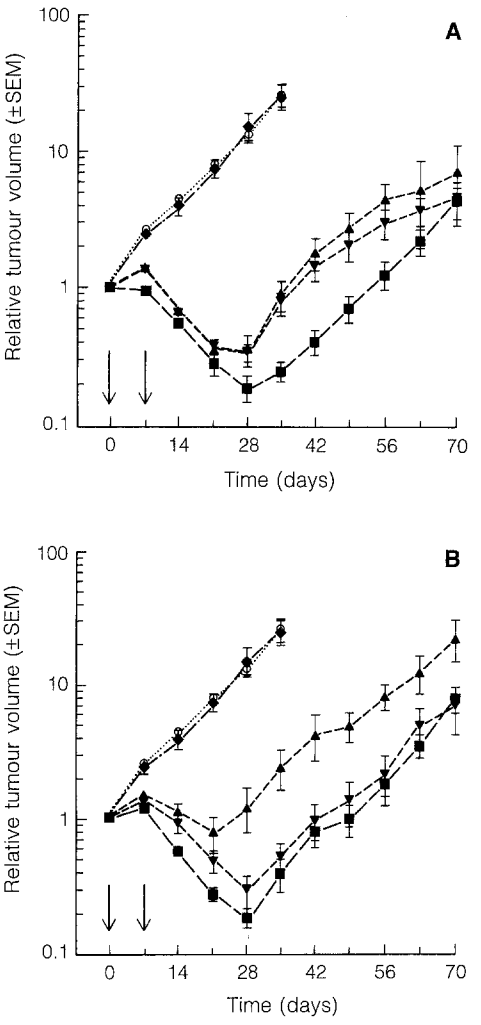

Fig. 5 A, B. Relative volumes of well-established s.c. OVCAR-3 xenografts grown in female nude mice after a conventional dose of platinum-containing drug (weekly × 2) injected alone (▲) and in combination with 200 mg/kg WR2721 given 5 min previously (▼) or with 200 mg/kg WR2721 given 5 min prior to a higher, equitoxic dose of platinum-containing drug (weekly × 2; ■) in comparison with those in untreated (○) and WR2721-treated (+) mice. **A** Cisplatin given at a 5-mg/kg (conventional) and an 8-mg/kg (high) dose (after [175]). **B** Carboplatin given at a 60-mg/kg (conventional) and a 90-mg/kg (high) dose (after [176])

ably due to the longer period of incubation of cisplatin and DNA used in the earlier studies [17, 62]. Platinum-protein interactions were hardly reversed by WR2721 or its main metabolites [173] (Table 1). Platinum-cysteine interactions could not be reversed by any of the WR compounds or by TS or DDTC. However, cisplatin-methionine interactions were rapidly reversed by DDTC and TS, whereas WR1065 was much slower in reversing this type of platinum-protein interaction and WR2721 and WR33278 hardly reversed this type of binding. This was confirmed by their capacity to restore the enzyme activity of fumarase after its inactivation by platination of the methionine residue in the active centre.

On the basis of these in vitro results, the prevention of cisplatin-induced damage by the thiol metabolite WR1065 is considered to be the most probable mechanism of protection. Because of the dramatic effect on cisplatin-DNA ad-

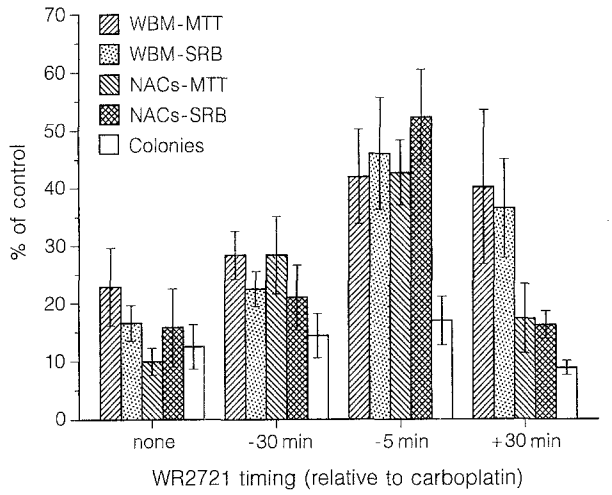

Fig. 6. Bone marrow function (% of control values ± SEM) of female BALB/c mice treated i.p. with 90 mg/kg carboplatin in the presence or absence of 200 mg/kg i.p. WR2721 at various administration times relative to carboplatin. In vitro proliferation of whole bone marrow (*WBM*) and non-adherent cells (*NACs*) was determined by both MTT and SRB staining. The clonogenic capacity of the bone marrow was determined by a bilayered soft-agar assay (after [176]).* Significantly different from the group treated with carboplatin alone according to Wilcoxon's rank-sum test (double-sided, 2 a $\leq$)

duct formation, interference with antitumour activity might be expected when the tumour is capable of efficiently dephosphorylating WR2721 and of taking up large amounts of the thiol metabolite WR1065. Thus, the preferential formation and uptake of WR1065 by non-tumour tissues appears to be crucial for the successful use of WR2721 in platinum-based chemotherapy. This was confirmed by the reduced cytostatic activity of cisplatin in the presence of WR1065 (in contrast to WR2721), in human ovarian cancer cells [174] (Fig. 3). Protection was better when WR1065 was incubated with the cancer cells concomitantly with cisplatin (co-incubation) as compared with incubation of the cells with WR1065 prior to a cisplatin exposure (pre-incubation). The protective effect of pre-incubation with WR1065 declined when the period of exposure to cisplatin was increased. Neither WR1065 nor WR2721 could protect cells from the cytostatic effect of cisplatin when the WR compounds were incubated with the cells 1 h after the cisplatin exposure. This effect confirms the hypothesis that the reversal of platinum-induced damage is not an important mechanism of protection.

Considering the fast uptake of WR1065 in (preferentially non-tumour) tissues and its subsequent rapid clearance from these tissues, an optimal administration scheme was suggested by the administration of WR2721 5 min prior to treatment with the platinum drug [171]. However, the reduction of cisplatin-induced nephrotoxicity in mice was the same (not improved) when WR2721 was given 5 min prior to cisplatin as compared with 30 min prior to cisplatin [175] (Fig. 4). WR2721 given 30 min after cisplatin did not protect mice at all from nephrotoxicity. This finding is in agreement with the hypothesis that the prevention of damage is the main mechanism of protection. In immuno-deficient (nude) mice bearing human ovarian carcinoma xenografts, WR2721 given 5 min prior to cisplatin did not interfere with the antitumour efficacy of cisplatin [175] Fig. 5A). This effect is in agreement with the hypothesis that WR2721 or its metabolites are not expected to inactivate a significant amount of the intact platinum drug in the circulation. The increased MTD of cisplatin given in combination with WR2721 resulted in an increased therapeutic efficacy.

Modulation of carboplatin-induced bone marrow toxicity by WR2721 was less obvious than its modulation of cisplatin-induced nephrotoxicity [176] (Fig. 6). Especially the late haemopoietic progenitor cells appeared to be partly protected by WR2721. The effect was more obvious when WR2721 was given 5 min prior to carboplatin than when it was used 30 min before or 30 min after the platinum drug. WR2721 given 5 min prior to carboplatin did not interfere with the tumour growth reduction in nude mice bearing human ovarian carcinoma xenografts [176] Fig. 5B). Again, this finding is in accordance with the hypothesis that WR2721 or its metabolites are not expected to inactivate a significant amount of the intact platinum drug in the circulation. On the contrary and quite surprisingly, WR2721 was shown to potentiate the tumour growth reduction induced by carboplatin. At the higher MTD of carboplatin, the increase in therapeutic efficacy appeared to be a little higher than that obtained with the conventional carboplatin dose plus WR2721.

In conclusion, WR2721 is a promising chemoprotective agent in reducing multiple side effects without interfering with antitumour efficacy. Various molecular aspects behind the selective modulation of cisplatin- and carboplatin-induced toxicities have been studied and the results extrapolated to the cellular and the in vivo level. This has generated new insights for a more rational and improved use of WR2721 in platinum-based chemotherapy. Clinical trials for the use of platinum antitumour drugs in combination with WR2721 in the treatment of head and neck cancer have recently been started, using these new insights together with previous experiences in clinical trials of cisplatin and WR2721.

References

1. Aamdal S, Fodstad O, Pihl A (1988) Sodium thiosulphate fails to increase the therapeutic index of intraveneously administered *cis*-diamminedichloroplatinum(II) in mice bearing murine and human tumors. Cancer Chemother Pharmacol 21: 129
2. Abe R, Akiyoshi T, Koba F, Tsuji H, Baba T (1988) Two-route chemotherapy using intra-arterial cisplatin and intravenous sodium thiosulphate, its neutralizing agent, for hepatic malignancies. Eur J Cancer Clin Oncol 24: 1671
3. Abe R, Akiyoshi T, Baba T (1990) Inactivation of *cis*-diamminedichloroplatinum(II) in blood by sodium thiosulphate. Oncology 47: 65
4. Abe R, Akiyoshi T, Baba T (1990) Two-route chemotherapy using cisplatin and its neutralizing agent, sodium thiosulphate, for intraperitoneal cancer. Oncology 47: 422
5. Allan SG, Smyth JF, Hay FG, Leonard RCF, Wolf CR (1986) Protective effect of sodium 2-mercaptoethanesulfonate on the gastrointestinal toxicity and lethality of *cis*-diamminedichloro-platinum(II). Cancer Res 46: 3569
6. Anderson ME, Naganuma A, Meister A (1990) Protection against cisplatin toxicity by administration of glutathione ester. FASEB J 4: 3251

7. Andrews PA, Howell SB (1990) Cellular pharmacology of cisplatin: perspectives on mechanisms of acquired resistance. Cancer Cells 2: 35
8. Baldew GS, Vermeulen NPE (1990) The role of lipid peroxidation in the nephrotoxicity of cisplatin. Ph.D. Thesis, ch 8, Free University, Amsterdam, The Netherlands
9. Baldew GS, Hamer CJA van den, Los G, Vermeulen NPM, Goeij JJM de, McVie JG (1989) Selenium-induced protection against *cis*-diamminedichloroplatinum(II) nephrotoxicity in mice and rats. Cancer Res 49: 3020
10. Baldew GS, McVie GJ, Valk MA van der, Los G, Goeij JJM de, Vermeulen NPE (1990) Selective reduction of *cis*-diamminedichloroplatinum(II) nephrotoxicity by ebselen. Cancer Res 50: 7031
11. Barry MA, Behnke CA, Eastman A (1990) Activation of programmed cell death (apoptosis) by cisplatin, other anticancer drugs, toxins and hyperthermia. Biochem Pharmacol 40: 2353
12. Basinger MA, Jones MM, Gilbreath SG, Walker EM, Fody EP, Mayhue MA (1989) Dithiocarbamate-induced biliary platinum excretion and the control of *cis*-platinum nephrotoxicity. Toxicol Appl Pharmacol 97: 279
13. Basinger MA, Jones MM, Holscher MA (1990) L-Methionine suppresses pathological sequelae of *cis*-platinum in the rat. Fundam Appl Toxicol 14: 568
14. Basinger MA, Jones MM, Holscher MA (1990) L-Methionine antagonism of *cis*-platinum nephrotoxicity. Toxicol Appl Pharmacol 103: 1
15. Berry JM, Jacobs C, Sikic B, Halsey J, Borch RF (1990) Modification of cisplatin toxicity with diethyldithiocarbamate. J Clin Oncol 8: 1585
16. Bird JE, Walser MM, Quebbemann J (1984) Protective effect of organic cation transport inhibitors on *cis*-diamminedichloroplatinum-induced nephrotoxicity. J Pharmacol Exp Ther 231: 752
17. Bodenner DL, Dedon PC, Keng PC, Borch RF (1986) Effect of dithiocarbamate on *cis*-diamminedi-chloroplatinum(II)-induced cytotoxicity, DNA cross-linking, and y-glutamyl transpeptidase inhibition. Cancer Res 46: 2745
18. Bodenner DL, Dedon PC, Keng PC, Katz JC, Borch RF (1986) Selective protection against *cis*-diamminedichloroplatinum(II)-induced toxicity in kidney, gut, and bone marrow by diethyldithiocarbamate. Cancer Res 46: 2751
19. Boelrijk AEM, Boogaard PJ, Lempers ELM, Reedijk J (1991) Regeneration experiments of the platinated enzyme fumarase by sodium diethyldithiocarbamate, thiourea and sodium thiosulphate. J Inorg Biochem 41: 17
20. Boogaard PJ, Slikkerveer A, Nagelkerke F, Mulder GJ (1991) The role of metallothionein in the reduction of cisplatin-induced nephrotoxicity by Bi^{3+}-pretreatment in the rat in vivo and in vitro. Biochem Pharmacol 41: 369
21. Boogerd W, Bokkel Huinink WW ten, Dalesio O, Hoppenbrouwers WJJF, Sande JJ van der (1990) Cisplatin-induced neuropathy: central, peripheral and autonomic nerve involvement. J Neuroncol 9: 255
22. Bompart G (1989) Cisplatin-induced changes in cytochrome P-450, lipid peroxidation and drug-metabolizing activities in rat kidney cortex. Toxicol Lett 48: 193
23. Borch RF, Markman M (1989) Biochemical modulation of cisplatin toxicity. Pharmacol Ther 41:371
24. Borch RF, Pleasants ME (1979) Inhibition of *cis*-platinum nephrotoxicity by diethyldithiocarbamate in a rat model. Proc Natl Acad Sci USA 76: 6611
25. Borch RF, Katz JC, Lieder PH, Pleasants ME (1980) Effect of diethyldithiocarbamate on tumor response to *cis*-platinum in a rat model. Proc Natl Acad Sci USA 77: 5441
26. Brabec V, Kleinwachter V, Butour JL, Johnson NP (1990) Biophysical studies of the modification of DNA by antitumour platinum coordination complexes. Biophys Chem 35: 129
27. Brady HR, Kone BC, Stromski ME, Zeidel ML, Giebisch G, Gullans SR (1990) Mitochondrial injury: an early event in cisplatin toxicity to renal proximal tubules. Am J Physiol 258: F1181
28. Brown DQ, Graham WJ, McKenzie LJ, Pittock JW, Shaw LM (1988) Can WR2721 be improved upon? Pharmacol Ther 39: 157
29. Bull JMC, Strebel FR, Sunderland BA, Bulger RE, Edwards M, Siddik Z, Newman RA (1988) *o*-(*B*-Hydroxyethyl)-rutoside-mediated protection of renal injury associated with *cis*-diamminedichloroplatinum(II)/hyperthermia treatment. Cancer Res 48: 2239
30. Calabro-Jones PM, Aguilera JA, Ward JF, Smoluk GD, Fahey RC (1988) Uptake of WR 2721 derivatives by cells in culture: identification of the transported form of the drug. Cancer Res 48: 3634
31. Catino JJ, Francher DM, Schurig JE (1986) Evaluation of *cis*-diamminedichloroplatinum(II) combined with metoclopramide or sodium thiosulphate on L1210 leukemia in vitro and in vivo. Cancer Chemother Pharmacol 18: 1
32. Daley-Yates PT, McBrien DCH (1982) The mechanism of renal clearance of cisplatin (*cis*-diamminedichloroplatinum II) and its modification by furosemide and probenecid. Biochem Pharmacol 31: 2243
33. Daley-Yates PT, McBrien DCH (1984) Cisplatin metabolites in plasma: a study of their pharmacokinetics and importance in the nephrotoxic and antitumour activity of cisplatin. Biochem Pharmacol 33: 3063
34. Daley-Yates PT, McBrien DCH (1984) Enhancement of cisplatin nephrotoxiciy by probenecid. Cancer Treat Rep 68: 445
35. Daugaard G, Abildgaard U (1989) Cisplatin nephrotoxicity. Cancer Chemother Pharmacol 25: 1
36. Dedon PC, Borch RF (1987) Characterization of the reactions of platinum antitumor agents with biologic and nonbiologic sulphur-containing nucleophiles. Biochem Pharmacol 36: 1955
37. DeGregorio MW, Gandara DR, Holleran WM, Perez EA, King CC, Wold HG, Montine TJ, Borch RF (1989) High-dose cisplatin with diethyldithiocarbamate (DDTC) rescue therapy: preliminary pharmacologic observations. Cancer Chemother Pharmacol 23: 276
38. Den Engelse L (1991) Immunocytochemical analysis of platinum-DNA adducts. Proceedings 6th International Symposium on Platinum and other Metal Coordination Compounds in Cancer Chemotherapy, San Diego, January 23 – 26
39. Deray G, Dubois M, Beaufils H, Cacoub P, Anoucr M, Jaudon MC, Baumelou A, Jouanneau C, Jacobs C (1988) Effects of nifedipine on cisplatinum-induced nephrotoxicity in rats. Clin Nephrol 30: 146
40. De Witt LM, Jones TW, Moore L (1988) Stimulation of the renal endoplasmic reticulum calcium pump: a possible biomarker for platinate toxicity. Toxicol Appl Pharmacol 92: 157
41. Dible SE, Siddik ZH, Boxall FE, Harrap KR (1987) The effect of diethyldithiocarbamate on the haematological toxicity and antitumour activity of carboplatin. Eur J Cancer Clin Oncol 23: 813
42. Di Re F, Bohm S, Oriana S, Spatti GB, Zunino F (1990) Efficacy and safety of high-dose cisplatin and cyclophosphamide with glutathione protection in the treatment of bulky advanced epithelial ovarian cancer. Cancer Chemother Pharmacol 25: 355
43. Dobyan DC, Bull JMC, Strebel FR, Sunderland BA, Bulger RE (1986) Protective effects of *O*-(*b*-hydroxyethyl)-rutoside on cis-platinum-induced acute renal failure in the rat. Lab Invest 55: 557
44. Dornish JM, Pettersen EO (1990) Requirement of a reactive aldehyde moiety for aldehyde-mediated protection against *cis*-diamminedichloroplatinum(II)-induced cell inactivation. Biochem Pharmacol 39: 309
45. Dorr RT, Soble MJ (1988) Cimetidine enhances cisplatin toxicity in mice. J Cancer Res Clin Oncol 114: 1
46. Doz F, Berens ME, Spencer DR, Dougherty DV, Rosenblum ML (1991) Experimental basis for increasing the therapeutic index of carboplatin in brain tumor therapy by pretreatment with WR-compounds. Cancer Chemother Pharmacol 28: 308
47. Eastman A, Schulte N, Sheibani N, Sorensen CM (1988) Mechanisms of resistance to platinum drugs. In: Nicolini M (ed) Platinum and other metal coordination compounds in cancer chemotherapy. Martinus Nijhoff, Boston Dordrecht Lancaster, p 178
48. Elferink F, Vijgh WJF van der, Klein I and Pinedo HM (1986) Interaction of cisplatin and carboplatin with sodium thiosulphate: reaction rates and protein binding. Clin Chem 32: 641

49. Evans RG, Wheatley C, Engel C, Nielsen J, Ciborowski LJ (1984) Modification of the bone marrow toxicity of *cis*-diamminedichloroplatinum(II) in mice by diethyldithiocarbamate. Cancer Res 44: 3686
50. Farnworth PG, Stone F, Rose IAG, Hillcoat BL (1989) Effects of cisplatinum on metallothionein levels in mouse liver and kidney and in L1210 and L1210/PDD cell lines. Proc Am Ass Cancer Res 30: 2090
51. Fichtinger-Schepman AMJ, Veer JL van der, Hartog JHJ den, Lohman, PHM, Reedijk J (1985) Adducts of the antitumor drug *cis*-diamminedichloroplatinum(II) with DNA: formation, identification and quantitation. Biochemistry 24: 707
52. Fichtinger-Schepman AMJ, Vendrik CPJ, Dijk-Knijnenburg WCM van, Jong WH de, Minnen ACE van der, Claessen AME, Velde-Visser SD van der, Groot G de, Wubs KL, Steerenberg PA, Schornagel J, Berends F (1989) Platinum concentrations and DNA-adduct-levels in tumors and organs of cisplatin-treated LOU/M rats inoculated with cisplatin-sensitive or -resistant immunoglobulin M immunocytoma. Cancer Res 49: 2862
53. Fichtinger-Schepman AMJ, Velde SD van der, Dijk-Knijnenburg WCM van, Oosterom AT van, Baan RA, Berends F (1990) Kinetics of the formation and removal of cisplatin-DNA adducts in human blood cells and tumor tissue of cancer patients receiving chemotherapy: comparison with in vitro adduct formation. Cancer Res 50: 7887
54. Gandara DR, Degregorio MW, Wold H, Wilbur BJ, Kohler M, Lawrence HJ, Deisseroth AB, George CB (1986) High-dose cisplatin in hypertonic saline: reduced toxicity of a modified dose schedule and correlation with plasma pharmacokinetics. A Northern California Oncology Group pilot study in non-small cell lung cancer J Clin Oncol 4: 1787
55. Gebbia V, Valenza R, Rausa L (1990) The in vitro effect of recombinant erythropoietin on *cis*-diamminedichloroplatinum-induced inhibition of murine erythroid stem cells. Anticancer Res 10: 1779
56. Gemba M, Nakatani E, Teramoto M, Nakano S (1987) Effect of cisplatin on calcium uptake by rat kidney cortical mitochondria. Toxicol Lett 38: 291
57. Gerritsen van der Hoop R, Koning P de (1988) Efficacy of the neuropeptide ORG.2766 in the prevention and treatment of cisplatin-induced neurotoxicity in rats. Eur J Cancer Clin Oncol 24: 637
58. Gerritsen van der Hoop R, Vecht CJ, Burg MEL van der, Elderson A, Boogerd W, Heimans JJ, Vries EP de, Houwelingen JC van, Jennekens FGI, Gispen WH, Neijt JP (1990) Prevention of cisplatin neurotoxicity with an ACTH(4–9) analogue in patients with ovarian cancer. N Engl J Med 322: 89
59. Glover D, Grabelsky S, Fox K, Weiler C, Cannon L, Glick J (1989) Clinical trials of WR-2721 and *cis*-platinum. Int J Radiat Oncol Biol Phys 16: 1201
60. Goel R, Cleary SM, Horton C, Kirmani S, Abramson IS, Kelly C, Howell SB (1989) Effect of sodium thiosulphate on the pharmacokinetics, toxicity of cisplatin. J Natl Cancer Inst 81: 1552
61. Goel R, Andrews PA, Pfeiffle CE, Abramson IS, Kirmani S, Howell SB (1990) Comparison of the pharmacokinetics of ultrafilterable cisplatin species detectable by derivatization with diethyldithiocarbamate or atomic absorption spectroscopy. Eur J Cancer 26: 21
62. Gonias SL, Oakley AC, Walther PJ, Pizzo SV (1984) Effects of diethyldithiocarbamate and nine other nucleophiles on the intersubunit protein cross-linking and inactivation of purified human α-macroglobulin by *cis*-diamminedichloroplatinum(II). Cancer Res 44: 5764
63. Gordon JA, Gattone VH (1986) Mitochondrial alterations in cisplatin-induced acute renal failure. Am J Physiol 250: F991
64. Green D, Schein P (1991) Evaluation of chemoprotection by ip WR2721 and oral WR151327 in mice. Proceedings 7th International Symposium on Chemical Modifiers of Cancer Treatment, Clearwater, p 236
65. Gringeri A, Keng PC, Borch RF (1988) Diethyldithiocarbamate inhibition of murine bone marrow toxicity caused by *cis*-diamminedichloroplatinum(II) or diammine(1,1-cyclobutanedicarboxylato)platinum(II). Cancer Res 48: 5708
66. Hamada T, Nishiwaki Y, Kodama T, Hayashibe A, Nukuriya N, Sasaki H, Morikawa T, Hirosawa T, Matsuyama T (1989) Prevention of renal toxicity of cisplatin by administration of bismuth subnitrate. Jpn J Cancer Chemother 16: 3587
67. Hardy JR, Tan S, Fryatt I, Wiltshaw E (1990) How nephrotoxic is carboplatin? Br J Cancer 61: 644
68. Hasuda K, Kobayashi H, Aoki K, Taniguchi S, Baba T (1989) Efficacy of two-route chemotherapy using *cis*-diamminedichloroplatinum(II) and its antidote, sodium thiosulphate, in combination with angiotensin II for rat liver tumor. Int J Cancer 44: 373
69. Hasuda K, Kobayashi H, Aoki K, Taniguchi S, Baba T (1990) Increased therapeutic effect on metastatic liver tumors in rats of two-route chemotherapy using *cis*-diamminedichloroplatinum(II) and its antidote, sodium thiosulphate, with temporary clamping of the abdominal aorta. Cancer Chemother Pharmacol 26: 181
70. Heidemann HT, Muller S, Mertins L, Stepan G, Hoffmann K, Ohnhaus EE (1989) Effect of aminophylline on cisplatin nephrotoxicity in the rat. Br J Pharmacol 97: 313
71. Hirosawa A, Niitani H, Hayashibara K, Tsuboi E (1989) Effects of sodium thiosulphate in combination therapy of *cis*-diamminedichloroplatinum and vindesine. Cancer Chemother Pharmacol 23: 255
72. Howell SB, Taetle R (1980) Effect of sodium thiosulphate on *cis*-diamminedichloroplatinum(II) toxicity and antitumour activity in L1210 leukemia. Cancer Treat Rep 64: 611
73. Howell SB, Pfeifle CE, Wung WE, Ohlson RA, Lucas WE, Yon JL, Green M (1982) Intraperitoneal cisplatin with systemic thiosulphate protection. Ann Intern Med 97: 845
74. Howell SB, Pfeifle CE, Wung WE, Ohlson RA (1983) Intraperitoneal *cis*-diamminedichloroplatinum(II) with systemic thiosulphate protection. Cancer Res 43: 1426
75. Howell SB, Kirmaani S, McClay EF, Kim S, Braly P, Plaxe S (1991) Intraperitoneal cisplatin-based chemotherapy for ovarian carcinoma. Semin Oncol 18: 5
76. Inoue M, Shimizu C, Shimizu H, Tanizawa O (1991) Neutralizing effect of sodium thiosulphate on antitumour efficacy of cisplatin for human carcinoma xenografts in nude mice. Gynecol Oncol 40: 34
77. Ishikawa M, Takayanagi Y, Sasaki K (1990) Enhancement of cisplatin toxicity by buthionine sulfoxime, a glutathione-depleting agent, in mice. Res Commun Chem Pathol Pharmacol 67: 131
78. Iwamoto Y, Kawano T, Uozumi J, Aoki K, Baba T (1984) Two-route chemotherapy using high-dose i.p. cisplatin and i.v. sodium thiosulphate, its antidote, for peritoneally disseminated cancer in mice. Cancer Treat Rep 68: 1367
79. Iwamoto Y, Kawano T, Ishizawa M, Aoki K, Kuroiwa T, Baba T (1985) Inactivation of *cis*-diamminedichloroplatinum(II) in blood and protection of its toxicity by sodium thiosulphate in rabbits. Cancer Chemother Pharmacol 15: 228
80. Jacobs C, Coleman CN, Rich L, Hirst K, Weiner MW (1984) Inhibition of *cis*-diamminedichloroplatinum(II) secretion by the human kidney with probenecid. Cancer Res 44: 3632
81. Jacobs C, Kaubisch S, Halsey J, Lum BL, Gosland M, Coleman CN, Sikic BI (1991) The use of probenecid as a chemoprotector against cisplatin nephrotoxicity. Cancer 67: 1518
82. Jones MM, Basinger MA (1989) Control of nephrotoxicity in the rat during repeated *cis*-platinum treatments. J Appl Toxicol 9: 229
83. Jones MM, Basinger MA (1989) Thiol and thioether suppression of *cis*-platinum-induced nephrotoxicity in rats bearing the Walker 256 carcinosarcoma. Anticancer Res 9: 1937
84. Jordan SW, Yuhas JM, Glick J (1982) Modulation of *cis*-platinum renal toxicity by the radioprotective agent WR 2721. Exp Mol Pathol 36: 297
85. Kaldor JM, Day NE, Petterson F et al (1990) Leukemia following chemotherapy for ovarian cancer. N Engl J Med 322: 1
86. Kempf SR, Ivankovic S (1987) Nephrotoxicity and carcinogenic risk of *cis*-platin (cisplatin) prevented by sodium 2-mercaptoethanesulfonate (mesna): experimental results. Cancer Treat Rev 14: 365
87. Kiyozumi M, Inoue T, Kojima S Hidaka S, Tsuruoka M (1991) Protection against *cis*-diamminedichloroplatinum-induced nephrotoxicity in rats by *N*-benzyl-D-glucamine dithiocarbamate. Toxicology 67: 41

88. Kizu R, Fukuda M, Yamamoto T, Miyazaki M (1990) Studies on the reaction products of cisplatin with sodium thiosulphate and their pharmacokinetic behavior in rabbits. J Pharmacobiodyn 13: S21
89. Knox RJ, Friedlos F, Lydall DA, Roberts JJ (1986) Mechanism of cytotoxicity of anticancer platinum drugs: evidence that *cis*-diamminedichloroplatinum(II) and *cis*-diammine(1,1-dicarboxylato-cyclobutane)platinum(II) differ only in the kinetics of their interaction with DNA. Cancer Res 46: 1972
90. Kobayashi H, Hasuda K, Aoki K, Taniguchi S, Baba T (1990) Systemic chemotherapy in tumor-bearing rats using high-dose *cis*-diamminedichloroplatinum(II) with low nephrotoxicity in combination with angiotensin II and sodium thiosulphate. Int J Cancer 45: 940
91. Kobayashi H, Hasuda K, Taniguchi S, Baba T (1991) Therapeutic efficacy of two-route chemotherapy using *cis*-diamminedichloroplatinum(II) and its antidote, sodium thiosulphate, combined with the angiotensin-II-induced hypertension method in rat uterine tumor. Int J Cancer 47: 893
92. Kojima S, Inoue T, Kiyozumi M, Shimada H (1990) Protective effect of *N*-benzyl-D-glucamine dithiocarbamate against *cis*-diamminedichloroplatinum-induced toxicity in gastrointestinal tract and bone marrow in rats. Chem Pharm Bull 38: 3127
93. Kramer RA (1989) Protection against cisplatin nephrotoxicity by prochlorperazine. Cancer Chemother Pharmacol 25: 156
94. Kuroiwa T, Aoki K, Taniguchi S, Hasuda K, Baba T (1987) Efficacy of two-route chemotherapy using *cis*-diamminedichloroplatinum(II) and its antidote, sodium thiosulphate, in combination with angiotensin II in a rat limb tumor. Cancer Res 47: 3618
95. Kuwahara A, Saito T, Nakashima K, Shigemitsu Y, Hirao E, Kobayashi M (1989) Clinical efficacy of two-route chemotherapy with cisplatin and thiosulphate for patients with peritonitis carcinomatosa. Jpn J Cancer Chemother 16: 2752
96. Leeuwenkamp OR, Vijgh WJF van der, Neijt JP, Pinedo HM (1990) Reaction kinetics of cisplatin and its monoaquated species with the (potential) renal protecting agents (di)mesna and thiosulphate. Estimation of the effect of protecting agents on the plasma and peritoneal AUCs of cisplatin. Biochem Pharmacol 27: 111
97. Lempers ELM, Reedijk J (1990) Reversibility of binding of cisplatin-methionine in proteins by diethyldithiocarbamate or thiourea: a study with model adducts. Inorg Chem 29: 217
98. Lerza R, Bogliolo G, Mencoboni M, Saviani A, Pannacciulli I (1988) Studies on hemotoxicity of cyclophosphamide, doxorubicin and *cis*-diamminedichloroplatinum(II) with sodium 2-mercaptoethane-sulfonate. Tumori 74: 333
99. Mangold DJ, Huelle BK, Miller MA, Geary RS, Sanchez-Barona OT et al (1990) Pharmacokinetics and disposition of WR-1065 in the rhesus monkey. Drug Metab Dispos 18: 281
100. Markman M, Cleary S, Pfeifle CE and Howell SB (1985) Nephrotoxicity of high-dose intracavitary cisplatin with intravenous thiosulphate protection. Eur J Clin Oncol 21: 1015
101. Markman M, Cleary S, Pfeifle CE, Howell SB (1985) High-dose intracavitary cisplatin with intravenous thiosulphate: low incidence of serious neurotoxicity. Cancer 56: 2364
102. Markman M, D'Acquistino R, Iannotti N, Kris M, Hakes T, Bajorin D, Bosl G, Reichmann B, Casper, E Magill G, Budnick A (1991) Phase-I trial of high-dose intravenous cisplatin with simultaneous intravenous sodium thiosulphate. J Cancer Res Clin Oncol 117: 151
103. Mayer RD, Lee K, Cockett ATK (1989) Improved use of buthionine sulfoximine to prevent cisplatin nephrotoxicity in rats. J Cancer Res Clin Oncol 115: 418
104. McGovern EP, Swynnerton NF, Steele PD, Mangold DJ (1984) HPLC assay for 2-(3-aminopropyl)-ethanethiol (WR1065) in plasma Int J Radiat Oncol Biol Phys 10: 1517
105. Millar BC, Siddik ZH, Millar JL, Jinks S (1985) Mesna does not reduce cisplatin induced nephrotoxicity in the rat. Cancer Chemother Pharmacol 15: 307
106. Mistry P, Harrap KR (1991) Historical aspects of glutathione and cancer chemotherapy. Pharmacol Ther 49: 125
107. Mistry P, Lee C, McBrien DCH (1989) Intracellular metabolites of cisplatin in the rat kidney. Cancer Chemother Pharmacol 24: 73
108. Montine TJ, Borch RF (1988) Quiescent LLC-PK1 cells as a model for *cis*-diamminedichloroplatinum(II) nephrotoxicity and modulation by thiol rescue agents. Cancer Res 48: 6017
109. Montine TJ, Borch RF (1990) Role of endogenous sulfur-containing nucleophiles in an in vitro model of *cis*-diamminedichloroplatinum(II)-induced nephrotoxicity. Biochem Pharmacol 39: 1751
110. Motzer RJ, Gulati SC, Crown J, Weisen SF, Bosl GJ (1991) A phase I trial of high-dose carboplatin + etoposide + dose escalation of cyclophosphamide with autologous bone marrow rescue in patients with refractory germ cell tumours. Proc Am Assoc Cancer Res 32: 1021
111. Muggia F (1991) Introduction: cisplatin update. Semin Oncol 18 [Suppl 3]: 1
112. Muller LJ, Gerritsen van der Hoop R, Moorer-van Delft CM, Gispen WH, Roubos EW (1990) Morphological and electrophysiological study of the effects of cisplatin and ORG.2766 on rat spinal ganglion neurons. Cancer Res 50: 2437
113. Murthy MS, Rao LN, Khandekar JD, Scanlon EF (1987) Enhanced therapeutic efficacy of cisplatin by combination with diethyldithiocarbamate and hyperthermia in a mouse model. Cancer Res 47: 774
114. Naganuma A, Satoh M, Imura N (1984) Effect of selenite on renal toxicity and antitumor activity of *cis*-diamminedichloroplatinum(II) in mice inoculated with Ehrlich ascites tumor cell. J Pharmacobiodyn 7: 217
115. Naganuma A, Satoh M, Koyama Y, Imura N (1985) Protective effect of metallothionein inducing metals on lethal toxicity of *cis*-diamminedichloroplatinum(II). Toxicol Lett 24: 203
116. Naganuma A, Satoh M, Imura N (1987) Prevention of lethal and renal toxicity of *cis*-diamminedichloroplatinum(II) by induction of metallothionein synthesis without compromising its antitumor activity in mice. Cancer Res 47: 983
117. Nechay BR, Neldon SL (1984) Characteristics of inhibition of human renal adenosine triphosphatases by cisplatin and chloroplatinic acid. Cancer Treat Rep 68: 1135
118. Ohe T, Tsuda S, Sakata Y, Taniwaki M, Misawa S, Abe T (1990) *cis*-Diamminedichloroplatinum(II)-induced sister chromatid exchanges and chromosome aberration formation in cultured human lymphocytes and their inhibition by sodium thiosulphate. Mutat Res 244: 279
119. Ohkawa K, Tsukada Y, Dohzono H, Koike K, Terashima Y (1988) The effects of co-administration of selenium on cisplatin-induced toxicity and antitumor activity. Br J Cancer 58: 38
120. Ormond T, Pratt I, Ryan MP (1988) Reduced nephrotoxicity in vivo and in vitro of cisplatin-methionine complex. Br J Pharmacol [Suppl] 95: 584
121. Osman NM, Copley MP, Litterst CL (1984) Amelioration of cisplatin-induced nephrotoxicity by the diuretic acetazolamide in F344 rats. Cancer Treat Rep 68: 999
122. Ozols RF (1989) Cisplatin dose intensity. Semin Oncol 16; 22
123. Pannacciulli IM, Lerza RA, Bogliolo GV, Mencoboni MP, Saviane AG (1989) Effect of diethyldithiocarbamate on toxicity of doxorubicin, cyclophosphamide and *cis*-diamminedichloroplatinum(II) on mice haemopoietic progenitor cells. Br J Cancer 59: 371
124. Paredes J, Hong WK, Felder TB, Dimery IW, Choksi AJ, Newman RA, Castellanos AM, Robbins KT, McCarthy K, Atkinson N, Kramer AM, Hersh EM, Goepfert H (1988) Prospective randomized trial of high-dose cisplatin and fluorouracil infusion with or without sodium diethyldithiocarbamate in recurrent and/or metastatic squamous cell carcinoma of the head and neck. J Clin Oncol 6: 955
125. Parker RJ, Eastman A, Bostick-Bruton F, Reed E (1990) Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin-DNA lesions and reduced drug accumulation. J Clin Invest 87: 772
126. Parker RJ, Gill I, Tarone R, Vionnet J, Grunberg S, Muggia F, Reed E (1991) Platinum-DNA damage in leucocyte DNA of patients receiving cisplatin and carboplatin chemotherapy, measured by atomic absorption spectrometry. Carcinogenesis 12: 1253

127. Pera MF, Zook BC, Harder HC (1979) Effects of mannitol or furosemide diuresis on the nephrotoxicity and physiological disposition of *cis*-dichlorodiammineplatinum(II) in rats. Cancer Res 39: 1269
128. Perez EA, Hesketh PJ, Gandara DR (1991) Serotonin antagonists in the management of cisplatin-induced emesis. Semin Oncol 18: 73
129. Perez RP, Hamilton TC, Ozols RF (1990) Resistance to alkylating agents and cisplatin: insights from ovarian carcinoma model systems. Pharmacol Ther 48: 19
130. Quazi R, Chang AYC, Borch RF, Montine T, Dedon PC, Loughner J, Bennett JM (1988) Phase I clinical and pharmacokinetic study of diethyldithiocarbamate as a chemoprotector from toxic effects of cisplatin. J Natl Cancer Inst 80: 1486
131. Reed E, Yuspa SH, Zwelling L, Ozols RF, Poirier MC (1986) Quantitation of *cis*-diamminedichloroplatinum(II) (cisplatin)-DNA intrastrand adducts in testicular and ovarian cancer patients receiving cisplatin chemotherapy. J Clin Invest 77: 545
132. Reed E, Ozols RF, Tarone R, Yuspa SH, Poirier MC (1987) Platinum-DNA adducts in leucocyte DNA correlate with disease response in ovarian cancer patients receiving platinum-based chemotherapy. Proc Nat Acad Sci USA 84: 5024
133. Reedijk J (1987) The mechanism of action of platinum antitumor drugs. Pure Appl Chem 59: 181
134. Reeves PG, Saari JT (1990) Effect of *cis*-diamminedichloroplatinum(II) on metallothionein induction and trace element metabolism in rats fed different amounts of dietary zinc. J Nutr Biochem 1: 374
135. Reznik LV, Myazina EM, Shakchmatova EI, Gambaryan SP, Brovtsyn VK, Natochin YV, Jones MM(1991) The prevention of cisplatin-induced renal dysfunction by hydroxyl-containing dithiocarbamates. Br J Cancer 63: 234
136. Roberts JJ, Vijgh WJF van der, Vermorken JB, Douple EB (1984) Cisplatin. In: Pinedo HM, Chabner BA (eds) Cancer chemotherapy annual. Elsevier, Amsterdam, pp 118–132
137. Rosenberg B, Van Camp L, Trosko JE, Mansour VH (1969) Platinum compounds: a new class of potent antitumour agents. Nature 222: 385
138. Rothenberg ML, Ostchega Y, Steinberg SM, Young RC, Hummel S, Ozols RF (1988) High dose carboplatin with diethyldithiocarbamate chemoprotection in treatment of women with relapsed ovarian cancer. J Natl Cancer Inst 80: 1488
139. Rothenberg ML, Ozols RF, Hamilton TC (1989) Cisplatin drug resistance. Cancer Bull 41: 48
140. Safirstein R, Winston J, Goldstein M, Moel D, Dikman S, Guttenplan J (1986) Cisplatin nephrotoxicity. Am J Kidney Dis VIII: 356
141. Satoh M, Naganuma A, Imura N (1989) Optimum schedule of selenium administration to reduce lethal and renal toxicities of *cis*-diamminedichloroplatinum(II) in mice. J Pharmacobiodyn 12: 246
142. Scanlon KJ, Kashani-Sabet M, Miyachi H, Sowers LC, Rossi J (1989) Molecular basis of cisplatin resistance in human carcinomas: model systems and patients. Anticancer Res 9: 1301
143. Schmalbach TK, Borch RF (1989) Diethyldithiocarbamate modulation of murine bone marrow toxicity induced by *cis*-diammine(cyclobutanedicarboxylato)platinum(II). Cancer Res 49: 6629
144. Schmalbach TK, Borch RF (1990) Mechanism of diethyldithiocarbamate modulation of murine bone marrow toxicity. Cancer Res. 50: 6218
145. Sharma RP (1984) Cisplatinum: effect of zinc acetate pretreatment on cellular uptake and interactions with cytosolic ligands. Toxicology 32: 75
146. Shaw LM, Bonner HS, Turrisi A, Norfleet AL, Kligerman M (1986) Measurement of *S*-2-(3-aminopropyl-amino)ethanethiol (WR1065) in blood and tissue. J Liq Chromatogr 9: 845
147. Shaw LM, Glover D, Turrisi A, Brown DQ, Bonner HS, Norfleet AL, Weiler C, Glick JH, Kligerman MM (1988) WR2721 pharmacokinetics. Pharmacol Ther 39: 195
148. Shea M, Koziol JA, Howell SB (1989) Kinetics of thiosulphate, a cisplatin neutralizer. Clin Pharmacol Ther 35: 419
149. Shea TC, Flaherty M, Elias A, Eder JP, Antman K, Begg C, Schnipper L, Frei E, Henner WD (1991) A phase I clinical and pharmacokinetic study of carboplatin and analogues: bone marrow support. J Clin Oncol 7: 651
150. Sleijfer DT, Offerman JJG, Mulder NH, Verweij M, Hem GK van der, Schraffordt Koops H, Meijer S (1987) The protective potential of the combination of verapamil and cimetidine on cisplatin-induced nephrotoxicity in man. Cancer 60: 2823
151. Sommer S, Thorling EB, Jakobsen A, Steiness A, Ostergaard K (1989) Can bismuth decrease the kidney toxic effect of *cis*-platinum? Eur J Clin Oncol 25: 1903
152. Sugihara K, Nakano S, Koda M Tanaka K, Fukuishi N, Gemba M (1987) Stimulatory effect of cisplatin on production of lipid peroxidation in renal tissues. Jpn J Pharmacol 43: 247
153. Sugiyama S, Hayakawa M, Kato T, Hanaki Y, Shimizu K, Ozawa T (1989) Adverse effects of antitumor drug cisplatin on rat kidney mitochondria: disturbances in glutathione peroxidase activity. Biochem Biophys Res Commun 159: 1121
154. Sundquist WI, Lippard SJ (1990) The coordination chemistry of platinum anticancer drugs and related compounds with DNA. Coord Chem Rev 100: 293
155. Suzuki CAM, Cherian MG (1990) The interactions of *cis*-diamminedichloroplatinum(II) with metallothionein and glutathione in rat liver and kidney. Toxicology 64: 113
156. Suzuki M, Ohwada M, Sekiguchi I, Tamada T (1989) Histopathological study regarding the protective effect of elastase on *cis*-platinum-induced renal toxicity. J Jpn Soc Cancer Ther 24: 941
157. Swynnerton NF, McGovern EP, Nino JA, Mangold DJ (1984) An improved HPLC assay for *S*-2-(3-aminopropyl)ethylphosphorothioate in plasma. Int J Radiat Oncol Biol Phys 10: 1521
158. Swynnerton NF, Huelle BK, McGovern EP, Mangold DJ (1986) Measurement of WR-1065 in plasma: preliminary pharmacokinetics in the beagle. Res Commun Chem Pathol Pharmacol 54: 255
159. Swynnerton NF, Huelle BK, Mangold DJ (1986) A method for the combined measurement of ethiofos and WR-1065 in plasma: application to pharmacokinetic experiments with ethiofos and its metabolites. Int J Radiat Oncol Biol Phys 12: 1495
160. Takada K, Takashima S, Nakamura K, Morimoto A, Inoue T, Fujimoto K, Murata K, Usuki N, Minakuchi K, Onoyama Y (1991) Two route chemotherapy by CDDP and STS against liver tumor. Jpn J Cancer Chemother 18: 57
161. Takahashi K, Seki T, Nishikawa K, Minamide S, Iwabuchi M, Ono M, Nagamine S, Horinishi H (1985) Antitumor activity and toxicity of serum-protein-bound platinum formed from cisplatin. Jpn J Cancer Res 76: 68
162. Takao S, Maenohara S, Yoshinaga A, Tokushige M, Hokita S, Aikou T, Nomura H, Shimazu S (1988) Two-route chemotherapy with cisplatin (i.p.) and its antidote sodium thiosulphate (i.v.) against carcinomatous peritonitis following alimentary tract cancers. J Jpn Soc Cancer Ther 23: 1696
163. Takizawa K, Harada M, Fujimaru J, Shima Y, Yokoo I, Ozaki I, Sato M, Iguchi T, Takeda Y (1990) The hypertensive effect induced with angiotensin II and the physiological and pharmacokinetic aspects of two-route chemotherapy using cisplatin and STS. Jpn J Cancer Chemother 17: 1569
164. Tay LK (1987) Characterization of cisplatin effects in the rabbit kidney and in renal proximal tubule cells in vitro. Proc Am Assoc Cancer Res 28: 1780
165. Ten Bokkel Huinink WW, Rodenhuis S, Schornagel JH, Dubbelman R, Koier I, Pinedo HM (1991) Carboplatin and growth factors for the bone marrow. Proceedings, 6th international Symposium on Platinum and Other Metal Compounds in Cancer Chemotherapy, San Diego v, p 31
166. Terheggen PMAB, Floot BGJ, Scherer E, Begg AC, Fichtinger-Schepman AMJ, Engelse L den (1987) Immunocytochemical detection of interaction products of *cis*-diamminedichloroplatinum(II) and *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) with DNA in rodent tissue sections. Cancer Res 47: 6719

167. Terheggen PMAB, Floot BGJ, Scherer E, Begg AC, Fichtinger-Schepman AMJ, Engelse L den (1988) Monitoring of interaction products of *cis*-diamminedichloroplatinum(II) and *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) with DNA in cells from cisplatin-treated cancer patients. Cancer Res 48: 5597
168. Terheggen PMAB, Emondt JY, Floot BGJ, Dijkman R, Schrier PI, Engelse L den, Begg AC (1990) Correlation between cell killing by *cis*-diamminedichloroplatinum(II) in six mammalian cell lines and binding of a *cis*-diamminedichloroplatinum(II)-DNA antiserum. Cancer Res 50: 3556
169. Terheggen PMAB, Begg AC, Emondt JY, Dubbelman R, Floot BGJ, Engelse L den (1991) Formation of interaction products of carboplatin with DNA in vitro and in cancer patients. Br J Cancer 63: 195
170. Thompson SW, Davis LE, Kornfeld M, Hilgers RD, Standefer JC (1984) Cisplatin neuropathy. Clinical, electrophysiologic, morphologic, and toxicologic studies. Cancer 54: 1269
171. Treskes M, Holwerda U, Klein I, Pinedo HM, Van der Vijgh WJF (1991) The chemical reactivity of the modulating agent WR2721 (ethiofos) and its main metabolites with the antitumor agents cisplatin and carboplatin. Biochem Pharmacol 42: 2125
172. Treskes M, Nijtmans LGJ, Fichtinger-Schepman AMJ, Van der Velde-Visser SD, Van der Vijgh WJF (1992) Effects of the modulating agent WR2721 and its main metabolites on the formation and stability of cisplatin/DNA adducts in vitro. Biochem Pharmacol 43: 1013
173. Treskes M, Holwerda U, Nijtmans LGJ, Pinedo HM, Van der Vijgh WJF (1992) Reversal of cisplatin-protein interactions by WR2721 and its main metabolites WR1065 and WR33278. Cancer Chemother Pharmacol 29: 467
174. Treskes M, Nijtmans LGJ, Fichtinger-Schepman AMJ, Van der Vijgh WJF (1992) Cytostatic activity of cisplatin in the presence of WR2721 and its thiol metabolite WR1065 in OVCAR03 human ovarian cancer cells as compared to V79 fibroblasts. Anticancer Res 12: 2261
175. Treskes M, Boven E, Holwerda U, Pinedo HM, Van der Vijgh WJF (1992) Time dependence of the selective modulation of cisplatin-induced nephrotoxicity by WR2721 (ethiofos) in the mouse. Cancer Res 52: 2257
176. Treskes M, Boven E, Loosdrecht AA van de, Wijffels JFAM, Cloos J, Peters GJ, Pinedo HM, Van der Vijgh WJF (1993) Effects of the modulating agent WR2721 on myelotoxicity and antitumour activity in carboplatin-treated mice. (submitted) v
177. Uozumi J, Litterst CL (1985) The effect of cisplatin on renal ATPase activity in vivo and in vitro. Cancer Chemother Phamacol 15: 93
178. Uozumi J, Sagiyama K, Aoki K, Iwamoto Y, Baba T (1983) Effectiveness of two-route chemotherapy using cisplatin and its antidote, sodium thiosulphate, on lifespan of rats bearing metastatic liver tumors. Cancer Treat Rep 67: 106
179. Uozumi J, Ishizawa M, Iwamoto Y, Baba T (1984) Sodium thiosulphate inhibits *cis*-diamminedichloroplatinum(II) activity. Cancer Chemother Pharmacol 13: 82
180. Utley JF, Seaver N, Newton GL, Fahey RC (1985) Pharmacokinetics of WR-1065 in mouse tissue following treatment with WR-2721. Int J Radiat Oncol Biol Phys 10: 1525
181. Van der Vijgh WJF (1991) Clinical pharmacokinetics of carboplatin. Clin Pharmacokinet 21: 242–261
182. Verma S, Stewart DJ, Maroun JA, Nair RC (1990) A randomized phase II study of cisplatin alone versus cisplatin plus disulfiram. Am J Clin Oncol 13: 119
183. Vermorken JB, Pinedo HM (1982) Gastrointestinal toxicity of *cis*-diamminedichloroplatinum(II): a review. Neth J Med 25: 275
184. Vermorken JB, Kapteijn TS, Hart AAM, Pinedo HM (1983) Ototoxicity of *cis*-diamminedichloroplatinum(II): influence of dose, schedule and mode of administration. Eur J Cancer Clin Oncol 19: 53
185. Von Hoff DD, Schilsky R, Reichert CM Reddick RL, Rozencweig M Young RC, Muggia FM (1979) Toxic effects of *cis*-diamminedichloroplatinum(II) in man. Cancer Treat Rep 63: 1527
186. Wagstaff AJ, Ward A, Benfield P, Heel RC (1989) Carboplatin A preliminary review of its pharmacodynamic and pharmacokinetic properties and its therapeutic efficacy in the treatment of cancer. Drugs 37: 162
187. Walker EM, Schaeffer RF, Henle KJ, Schmidt BJ, Gale GR, Cannon DJ, Jones MM, Moss AJ (1989) Combination and individual antitumor effects of hyperthermia, cisplatin, and selected dithiocarbamates. Ann Clin Lab Sci 19: 242
188. Wang D, Wang L Yang T, Zou Q (1990) The protective effect of selenite on cisplatin nephrotoxicity. Chin J Pharmacol Toxicol 4: 296
189. Wasserman TH, Phillips TL, Ross G, Kane LJ (1981) Differential protection against cytotoxic chemotherapeutic effects on bone marrow CFUs by WR-2721. Cancer Clin Trials 4: 3
190. Weiner MW, Jacobs C (1983) Mechanisms of cisplatin nephrotoxicity. Fed Proc 42: 2974–2978
191. Wesley Alden W, Repta AJ (1984) Exacerbation of cisplatin-induced nephrotoxicity by methionine. Chem Biol Interact 48: 121
192. Yuhas JM, Culo F (1980) Selective inhibition of the nephrotoxicity of *cis*-diamminedichloroplatinum(II) by WR-2721 without altering its antitumor properties. Cancer Treat Rep 64: 57
193. Yuhas JM, Spellman JM, Jordan SW, Pardini MC, Afzal SMJ, Culo F (1980) Treatment of tumours with the combination of WR-2721 and *cis*-diamminedichloroplatinum(II) or cyclophosphamide. Br J Cancer 42: 574
194. Zhen W, Link CJ, O'Connor PM, Bohr VA (1991) Formation and repair of cisplatin lesions in specific genes in cisplatin sensitive and resistant human ovarian cancer cell lines. Proc Am Assoc Cancer Res 32: 7
195. Zhong LF, Zhang JG, Ma SL, Xia YX (1990) Protection against cisplatin-induced lipid peroxidation and kidney damage by procaine in rats. Arch Toxicol 64: 599
196. Zunino F, Pratezi G, Micheloni A, Cavalletti E, Sala F, Tofanetti O (1989) Protective effect of reduced glutathione against cisplatin-induced renal and systemic toxicity and its influence on the therapeutic activity of the antitumor drug. Chem Biol Interact 70: 89