*Eur J Cancer Clin Oncol*, Vol. 23, No. 6, pp. 813–818, 1987.
Printed in Great Britain

0277-5379/87$3.00+0.00


# The Effect of Diethyldithiocarbamate on the Haematological Toxicity and Antitumour Activity of Carboplatin

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**Abstract**—*Carboplatin has recently been established as an effective agent in cancer chemotherapy. The dose limiting toxicity of this platinum complex is myelosuppression and, in this study, we have shown that through the use of diethyldithiocarbamate (DDTC), a protective thiol compound, the toxicity in rodents can be ameliorated and lethality prevented. DDTC protected against carboplatin-induced leucopoenia and anaemia when administered as a single bolus or as a multiple schedule incorporating three injections. With the single bolus, the effectiveness of this protection decreased as the lag time between carboplatin and DDTC administrations increased. Thrombocytopoenia was unaffected by DDTC. The growth profile of ADJ/PC6A tumour in mice was dependent on the timing of DDTC administration when given in combination with the platinum complex. The tumour growth delay, however, was unaffected by DDTC at any schedule. Multiple injections of DDTC increased the* $ED_{90}$ *for carboplatin from* 5.0 *to* 7.5 mg/kg. *The* $LD_{50}$ *value was increased from* 115 *to* 216 mg/kg *with the same DDTC schedule. Thus, DDTC improved the therapeutic index of carboplatin by* 25%. *A clinical role for the use of DDTC as a protective agent for carboplatin-induced toxicities is suggested.*

## INTRODUCTION

CISPLATIN (*cis*-dichlorodiammineplatinum II) is a well established agent in cancer chemotherapy [1]. Its clinical application, however, is restricted by the onset of several toxicities of which renal damage is the most severe [2]. Carboplatin (Paraplatin, JM8, CBDCA) was developed by Harrap *et al.* [3] as a non-nephrotoxic alternative. Clinical trials have indicated that the analogue is also highly active against several human tumours [4]. The dose-limiting toxicity has been ascribed to myelosuppression, particularly thrombocytopoenia [5], which may be responsible for the secondary anaemia in both man and animals [5, 6]. In addition to analogue development, attempts have been made to alleviate the side effects of cisplatin with sulphur nucleophiles, such as thiourea [7], thiosulphate [8], WR-2721 [9] and diethyldithiocarbamate (DDTC) [10, 11]. Of these, the chelating agent DDTC has shown particular promise in that protection is afforded to the kidney without compromising the antitumour activity [11, 12]. A further property of DDTC is that protection also extends to the bone marrow, with cisplatin-induced leucopoenia being significantly reduced [13–15]. Since the clinical limitation to the use of carboplatin is myelosuppression, we have investigated the use of DDTC as a protective agent against carboplatin-induced bone marrow toxicity in mice and rats. In addition, the effect of DDTC on antitumour activity hs been explored to establish its full potential in combination with carboplatin.

Accepted 8 December 1986.
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Supported in part by grants from the Cancer Research Campaign/Medical Research Council and the Johnson Matthey Research Centre. S.E.D. is also grateful to the Cancer Research Campaign for a studentship.

## MATERIALS AND METHODS

*Animals and chemicals*

Studies were carried out in female BALB/c− mice (18–23 g) and female Wistar rats (200–230 g). Body weights of animals were recorded where appropriate. Carboplatin and cisplatin were gifts from the Johnson Matthey Research Centre (Reading, U.K.) and were dissolved in 5% dextrose or 0.9% saline, respectively. The sodium salt of DDTC (Sigma) was dissolved in 0.9% saline.

*Determination of blood cell counts*

Mice received a single lethal dose (150 mg/kg; 10 ml/kg) of carboplatin i.v. (tail vein). DDTC was

administered i.p. at either a multiple dose (300 mg/kg; 10 ml/kg) schedule at 0, 2 and 4 hr, or as a single injection (750 mg/kg; 10 ml/kg) at 0, 2, 4 or 24 hr. Control animals received dextrose i.v. and either saline or DDTC i.p. at appropriate intervals. Blood was sampled from the lateral tail vein into a heparinized 25 μl micropipette (Corning, U.S.A.). The pipettes were sealed at one end with plasticene and centrifuged at 1300 ***g*** for 15 min for haematocrit determinations. Rats received i.v. (tail vein) either carboplatin (60 mg/kg) or cisplatin (6.5 mg/kg) at 10 ml/kg, followed by a single (750 mg/kg) or multiple (300 mg/kg) injection of DDTC i.p. Blood was sampled from the tail into microfuge tubes containing 5 units of heparin, and placed on ice. Blood cell counts were determined electronically [16].

*Determination of antitumour activity*

Fragments (1 $mm^3$) of ADJ/PC6A tumour were implanted s.c. into the right flank of female BALB/c− mice. Mice received cisplatin or carboplatin i.v. with or without DDTC i.p. on day 22 post-implantation, when tumour volumes had reached 3–4 $cm^3$. Volumes were calculated from caliper measurements as described by Corbett *et al.* [17]. Individual tumours were normalized to the tumour volume (100%) at the start of treatment (referred to hereafter as day 0). The tumour growth delay was calculated according to Begg [18] for each animal where tumour regrowth was evident. Briefly, the growth delay was determined by comparing treated mice with the appropriate controls on the basis of the time taken for the tumours to reach a volume of 250%. There was no significant difference in tumour growth rates in animals receiving saline i.v. in conjunction with saline or DDTC i.p.

*Determination of therapeutic index (TI)*

TI is defined as the ratio of $LD_{50}$ to $ED_{90}$. BALB/c− mice bearing ADJ/PC6A tumours received carboplatin i.v. at effective (2–8 mg/kg) and lethal (115–232 mg/kg) dose ranges. Saline or DDTC was administered i.p. at 0, 2 and 4 hr. Tumour growth inhibition was calculated from the ratio of the tumour volume of the treated group to that of the controls on the day of sacrifice of control animals (day 10 post-treatment). $ED_{90}$ values were obtained by probit analysis [19]. $LD_{50}$ values were determined from groups of 4 mice each, according to the procedures of Weil [20], all deaths having occurred by day 14.

## RESULTS

*Effect of DDTC on haematological toxicity*

In mice, carboplatin-induced anaemia and loss in body weight. Anaemia was most severe at 2–3 weeks after drug administration with the nadir occurring on day 17. The effects of DDTC on anaemia and body weight loss are shown in Table 1. There appeared to be a direct relationship between depression in haematocrit and weight loss. The degree of protection with DDTC varied according to its schedule of administration. Protection was maximal when DDTC was given as a single bolus at 0 hr or as a multiple schedule incorporating 3 or more injections. With a single dose of DDTC, protection became less effective as the lag time between carboplatin and DDTC administration increased. Thus, administration of DDTC at 24 hr as a single dose provided the least protection. However, when the multiple schedule was extended to include a 24 hr administration, no additional protection was conferred. Recovery from anaemia and body weight loss was rapid, with normal levels being re-established by days 24–26.

The effect of DDTC on haematological toxicity has been studied in greater detail in the rat. At the doses used, there were no survivors in the carboplatin (60 mg/kg, i.v.) alone group, whereas all animals survived in the cisplatin (6.5 mg/kg, i.v.) alone group. DDTC, however, prevented carboplatin-induced lethalities when given as a bolus or in multiple injections. In addition, DDTC reduced cisplatin-induced elevations in BUN levels on day 3 (83 ± 3 vs 63 ± 11 mg/100 ml plasma).

Leucopoenia in rats was induced by both drugs (Fig. 1A). DDTC inhibited drug-induced reduction in white cells. With carboplatin, inhibition was greater when DDTC was administered as multiple injections. Nadir of leucopoenia occurred on day 9 with the peak of leucocytosis on day 15. Levels returned to control values by 4 weeks. The temporal aspects of thrombocytopoenia were similar for both platinum drugs, the nadir occurring on days 7–9 (Fig. 1B). This toxicity was more severe with carboplatin (90% cell reduction) than with cisplatin (70% cell reduction). Multiple doses of DDTC provided total protection against cisplatin-induced platelet depression. In contrast, DDTC at both single and multiple schedules had minimal effect on carboplatin-induced thrombocytopoenia. Thromobocytosis occurred on day 18, and control values were regained by day 28. The day of nadir of carboplatin-induced anaemia coincided closely with the day of death, and it is proposed that the severe red cell reduction probably accounts for the lethalities (Fig. 1C). A single dose of DDTC marginally reduced the severity of anaemia, with maximum depression in cell counts being 75% of controls. Multiple doses of DDTC, however, provided greater protection and cell counts were reduced to only 40% of normal values.

*Table* 1. *Protection afforded by DDTC in mice against carboplatin-induced anaemia*

| Treatment schedules | Haematocrit on day 17 | % Loss in body weight on day 6 ($n = 4$) | Day of death |
|---|---|---|---|
| Controls | 0.57 ± 0.05 (4) | 1.9 ± 1.8 | — |
| Carboplatin | — | 25.2 ± 3.8 | 6–7 |
| + DDTC 0 hr | 0.45 ± 0.07 (4) | 5.5 ± 2.2 | — |
| 2 hr | 0.09, 0.24 | 12.0 ± 1.3 | 11 |
| 4 hr | 0.08, 0.18 | 15.9 ± 3.8 | 11 |
| 24 hr | 0.08 | 19.3 ± 2.8 | 10 |
| 0, 2, 4 hr | 0.40 ± 0.01 | 2.8 ± 4.0 | — |
| 0, 2, 4, 24 hr | 0.34 ± 0.05 (4) | 9.0 ± 4.2 | — |
| 2, 4, 24 hr | 0.23 ± 0.09 (4) | 9.0 ± 4.5 | — |
| 2, 4 hr | 0.18 ± 0.08 (3) | 14.3 ± 5.0 | 14 |

Results are nadir values and reported as means ± SE ($n$ given in parentheses) or as individual values. Controls received saline and DDTC at time 0. Animals received a lethal dose of carboplatin (150 mg/kg, i.v.) with either a single (750 mg/kg, i.p.) or a multiple (300 mg/kg, i.p.) injection of DDTC. Control values were similar for either single or multiple DDTC schedules.

*Effect of DDTC on antitumour activity*

The temporal aspects of the characteristic response of the ADJ/PC6A tumour to cisplatin and carboplatin are shown in Fig. 2. A dose of 9 mg/kg carboplatin or 0.9 mg/kg cisplatin induced a rapid response resulting in 'cures' by days 10–12. With lower doses, it was possible to determine tumour growth delay, which, for instance, was 13 days for both 0.3 mg/kg cisplatin and 3 mg/kg carboplatin. The effect of DDTC on the antitumour activity of the platinum complexes, at either the single or multiple schedule, was tested at doses approximating the $ED_{90}$. Results of this study are shown in Fig. 3. The temporal aspects of the antitumour response were similar in cisplatin-treated mice with and without the multiple DDTC treatment (Fig. 3a). This DDTC schedule, in combination with carboplatin, produced a response–time profile which was different from that with carboplatin alone (Fig. 3b). Also, with a single injection of DDTC together with carboplatin the growth profile was dependent on the timing of DDTC administration. The tumour growth delay, however, appeared to be independent of the DDTC schedule employed, and was similar to the group treated only with carboplatin (Table 2).

The DDTC-induced dose modification for carboplatin was determined for both $ED_{90}$ and $LD_{50}$ values, in order to ascertain the effect on the TI (Table 3). The $ED_{90}$ for carboplatin with and without DDTC was 7.5 and 5.0 mg/kg, respectively, giving a modification factor of 1.5. The respective $LD_{50}$ values were 216 and 115 mg/kg, a 1.9-fold increase in the dose. Thus, DDTC improved the TI from 23 to 29.

## DISCUSSION

The use of a protective agent which is specific for normal tissues without a compromising effect on the tumour is of significant importance in cancer chemotherapy. Many sulphur nucleophiles (e.g. thiourea, thiosulphate, WR-2721, penicillamine and DDTC) have been incorporated as protective agents against cisplatin-induced toxicities [7–10]. However, of these existing alternatives, DDTC appears to be the most promising. This chelating agent not only affords protection in rodents against cisplatin-induced renal, gastrointestinal and bone marrow toxicities [12, 18, 19] but is devoid of any substantial effect on the antitumour activity against L1210 [21] and P388 [13] leukaemias, B16 melanoma, Lewis lung and colon 26 tumours [13] in mice although some compromise is evident using a mammary tumour [12]. Lack of effect of DDTC in combination with carboplatin against the P388 leukaemia has also been demonstrated [13].

In the present study, the protective effects of DDTC in cisplatin-induced leucopoenia have not only been confirmed, but we have also demonstrated that anaemia and thrombocytopoenia are ablated with the DDTC schedule employed. In addition, DDTC has demonstrated an ability to protect against carboplatin-induced toxicity. Protection was specific in that the severity of leucopoenia and anaemia reduced, but thrombocytopoenia was unaffected. The explanation for the differential effect

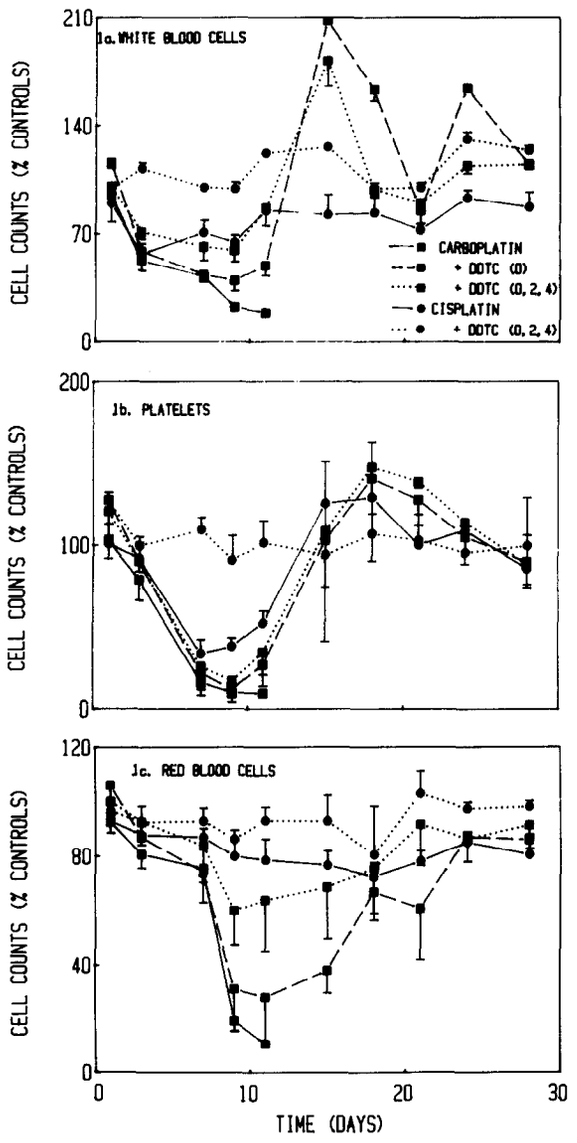


*Fig. 1. The effect of DDTC on carboplatin and cisplatin-induced white and red cell and platelet depression in rats. Results are expressed as means ± SE,* n = 4. *DDTC was administered at* 0 hr (*DDTC* 0) *or at* 0, 2 *and* 4 hr (*DDTC* 0, 2, 4) *following carboplatin* (60 mg/kg, *i.v.*) *or cisplatin* (6.5 mg/kg, *i.v.*). *Carboplatin induced* 100% *lethality on day* 11.

of DDTC on thrombocytopoenia caused by cisplatin and carboplatin is unknown, although it may involve differences in the recovery of precursor cells in the bone marrow.

Since anaemia has been reported as the dose-limiting toxicity of carboplatin in rats [6], amelioration of the anaemia may account for rodents surviving a lethal dose of carboplatin. This anaemia has previously been postulated to be due to haemorrhaging as a result of thrombocytopoenia [22]. Interestingly, in the present study, DDTC reduced the severity of anaemia in the absence of any significant effect on thrombocytopoenia. The slight improvement in platelet numbers with the DDTC-carboplatin combination, however, may be sufficient to afford some protection against anaemia.

A chemical interaction between cisplatin and DDTC has been demonstrated *in vitro* [23]. This reaction is assumed to occur between carboplatin and the thiol. The chemical interaction, however, does not completely neutralize the antitumour effects of both platinum complexes when administered simultaneously with DDTC. Indeed, for optimal protection, it was necessary to administer DDTC and carboplatin simultaneously. In contrast, the optimal lag time between DDTC and cisplatin administration has been reported as 1–2 hr to minimize the rise in BUN levels [11] and 2–5 hr for protection of leucopoenia [15]. We have demonstrated that similar protection is afforded against the haematological toxicity when DDTC is given as multiple injections at 0, 2 and 4 hr after cisplatin administration, although renal protection at this schedule may not be optimal. The greater inhibition of the development of carboplatin-induced anaemia in the rat with the multiple DDTC schedule as compared with a single bolus of DDTC may relate to more favourable pharmacokinetics, in that exposure of the target sites to the thiol would be prolonged.

With multiple DDTC administration, both the $LD_{50}$ and $ED_{90}$ of carboplatin were increased. The 90% increase in the $LD_{50}$ value for this combination compares favourably with the value of 120% reported for cisplatin and DDTC in mice, although in this case DDTC was administered as a single bolus [15]. The modification in the $ED_{90}$ value for the cisplatin-DDTC combination has not been previously reported. The data of Borch *et al.* [12] with a mammary tumour suggests that DDTC compromised the antitumour activity of cisplatin, this compromise being similar to that observed in the present study with the ADJ/PC6A tumour using carboplatin. This would suggest that the improvement in DDTC in the TI value would be similar for the two platinum complexes. However, if tumour growth delay is considered then DDTC has negligible effects on the antitumour activities of carboplatin, resulting in a two-fold improvement in the TI.

In conclusion, DDTC improves the therapeutic index of carboplatin and, therefore, this combination could be exploited clinically in cancer chemotherapy.

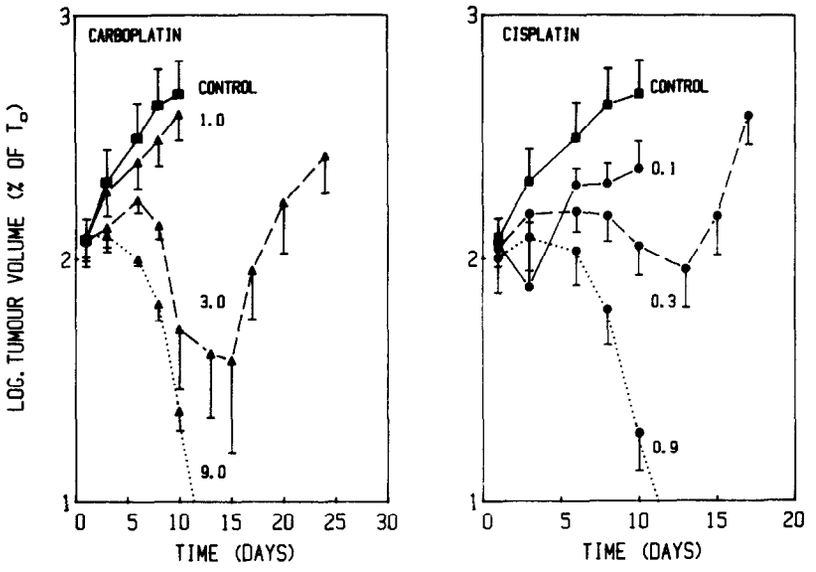


*Fig. 2. Growth of ADJ/PC6A tumour in mice receiving carboplatin or cisplatin. Results are expressed as means ± SE,* n = 5. *Doses in* mg/kg, *given i.v., are indicated on the curves. Controls were sacrificed on day* 10 *post treatment. At high doses, tumours were not palpable after day* 10 *and there was no reappearance of the tumour within the* 60 *day period. These were classified as 'cures'.*

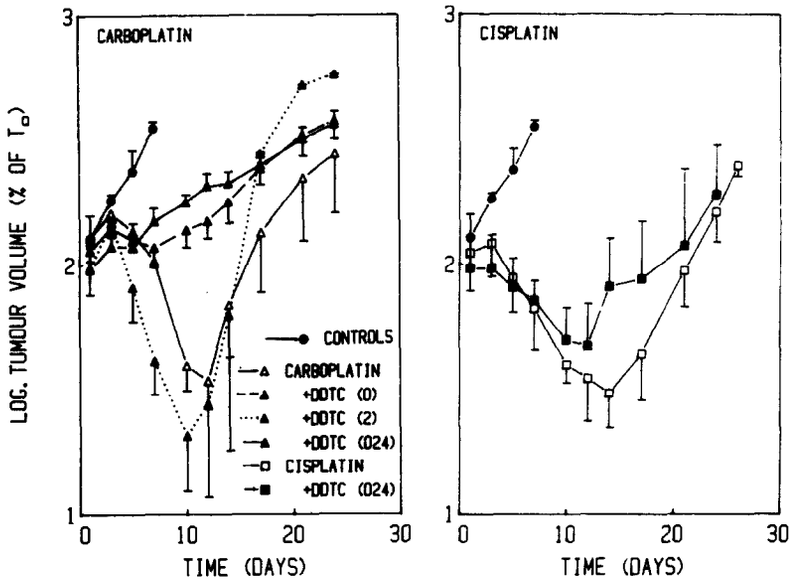


*Fig. 3. Growth of ADJ/PC6A tumour in mice receiving carboplatin or cisplatin with and without DDTC. Results are expressed as means ± SE or as individual values. 'Cures' have been excluded from data points. See Table 2 for number of 'cures' in each group. DDTC was administered at* 0 hr (*DDTC* 0), 2 hr (*DDTC* 2) *or* 0, 2 *and* 4 hr (*DDTC* 0, 2, 4) *after carboplatin* (4 mg/kg, *i.v.*) *or cisplatin* (0.6 mg/kg, *i.v.*).

## REFERENCES

1. Prestayko AW, D'Aoust JC, Issell BF, Crooke ST. Cisplatin (*cis*-diamminedichloroplatinum II). *Cancer Treat Rep* 1979, **6**, 17–39.
2. Krakoff IH. Nephrotoxicity of *cis*-dichlorodiammine-platinum II. *Cancer Treat Rep* 1979, **63**, 1527–1532.
3. Harrap KR, Jones M, Wilkinson CR *et al.* Antitumour, toxic and biochemical properties of cisplatin and eight other platinum complexes. In: Prestayko AW, Crooke ST, Carter SK, eds. *Cisplatin: Current Status and New Developments*. New York, Academic Press, 1980, 193–212.
4. Von Hoff DD, Rozencweig M. *cis*-Diammine-dichloroplatinum (II). A metal complex with significant antitumour activity. *Adv Pharmacol Chemother* 1979, **16**, 273–277.
5. Calvert AH, Harland SJ, Newell DR *et al.* Early clinical studies with *cis*-diammine-1,1-cyclobutane dicarboxylate platinum (II). *Cancer Chemother Pharmacol* 1982, **9**, 140–147.

*Table 2. Tumour growth delay in mice receiving carboplatin or cisplatin*

| Treatment schedule | Tumour growth delay (days) | No. cures |
|---|---|---|
| Carboplatin | 16.8± 3.8 (3) | 2 |
| + DDTC 0 hr | 13.6 ± 2.4 (4) | 1 |
| 2 hr | 12.0 | 4 |
| 0, 2, 4 hr | 13.4 ± 2.2 (5) | 0 |
| Cisplatin | 19.7 ± 0.9 (3) | 2 |
| + DDTC 0, 2, 4 hr | 17.8 ± 4.2 (3) | 2 |

Results are reported as means ± SE (*n* given in parentheses) or as individual values. 'Cures' have been excluded from calculations.
Each group contained 5 mice.
Doses are as specified in Fig. 3.

*Table 3. Therapeutic index of carboplatin and modification by DDTC*

| Parameter | Carboplatin | Carboplatin + DDTC | Dose modification factor (DMF) |
|---|---|---|---|
| $ED_{90}$ | 5.0 (4.2–5.8) | 7.5 (6.5–8.6) | 1.5 |
| $LD_{50}$ | 115 (114–116) | 216 (201–232) | 1.9 |
| TI | 23 | 29 | 1.25 |

The DMF is defined as the ratio of carboplatin and DDTC to carboplatin. Results for $LD_{50}$ and $ED_{90}$ are expressed as mean values (mg/kg) with 95% confidence limits in parentheses ($n$ = 4 per dose level).
DDTC (300 mg/kg, i.p.) was administered at 0, 2 and 4 hr after carboplatin.


6. Siddik ZH, Newell DR, Boxall FE, Jones M, McGhee KG, Harrap KR. Biliary excretion, renal handling and red cell uptake of cisplatin and CBDCA in animals. In: Hacker MP, Douple EB, Krakoff IH, eds. *Platinum Coordination Complexes in Cancer Chemotherapy*. Boston, Martinus Nijhoff, 1984, 90–102.
7. Filipski J, Kohn KW, Prather R, Bonner WM. Thiourea reverses crosslinks and restores biological activity in DNA treated with dichlorodiaminoplatinum II. *Science* 1979, **204**, 181–183.
8. Howell SB, Taetle R. Effect of sodium thiosulphate on *cis*-dichlorodiammine platinum (II) toxicity and antitumour activity in L1210 leukaemia. *Cancer Treat Rep* 1980, **64**, 611–616.
9. Yuhas JM, Culo F. Selective inhibition of *cis*-dichlorodiammine platinum II by WR-2721 without altering its antitumour properties. *Cancer Treat Rep* 1980, **64**, 57–64.
10. Gale GR, Atkins LM, Walker EM. Further evaluation of diethyldithiocarbamate as an antagonist of cisplatin toxicity. *Ann Clin Lab Sci* 1982, **12**, 345–355.
11. Borch RF, Pleasant ME. Inhibition of cisplatin nephrotoxicity by diethyldithiocarbamate rescue in a rat model. *Proc Natn Acad Sci U.S.A.* 1979, **76**, 6611–6614.
12. Borch RF, Katz JC, Lieder PH, Pleasants ME. Effect of diethyldithiocarbamate on tumour response to cisplatin in a rat model. *Proc Natn Acad Sci U.S.A.* 1980, **77**, 5441–5444.
13. Bodenner DL, Dedon PC, Keng DC, Katz JC, Borch RF. Selective protection against *cis*-diamminedichloroplatinum (II)-induced toxicity in kidney, gut and bone marrow by diethyldithiocarbamate. *Cancer Res* 1986, **46**, 2751–2755.
14. Gringeri A, Borch RF. Effect of DDTC on bone marrow cells treated with *cis*-diamminedichloroplatinum and its analogues. *Proc Am Ass Cancer Res* 1984, **25**, 371.
15. Evans RG, Wheatley C, Engel C, Nielsen J, Ciborowski LJ. Modification of bone marrow toxicity of *cis*-diamminedichloroplatinum (II) in mice by diethyldithiocarbamate. *Cancer Res* 1984, **44**, 3686–3690.
16. Delaney JW, Garraty G. *Handbook of Haematological and Blood Transfusion Techniques*. London, Butterworths, 1969.
17. Corbett TH, Roberts BJ, Trader MW, Laster WR, Griswold DP, Schabel FM Jr. Response of transplantable tumours of mice to anthracenedione derivatives. *Cancer Treat Rep* 1982, **66**, 1187–1200.
18. Begg AC. Analysis of growth delay data. Potential pitfalls. *Br J Cancer* 1980, **41**, 93–103.
19. Litchfield JR, Wilcoxan F. A simplified method of evaluating dose effect experiments. *J Pharmacol Exp Ther* 1949, **96**, 99–113.
20. Weil CS. Tables for convenient calculation of median effective dose ($LD_{50}$ and $ED_{90}$) and instructions in their use. *Biometrics* 1952, **8**, 249–263.
21. Khandekar JD. Improved therapeutic index of *cis*-diamminedichloroplatinum by diethyldithiocarbamate in rodents. *Res Commun Chem Pathol Pharmacol* 1982, **40**, 55–66.
22. Siddik ZH, Boxall FE, Harrap KR. Haematological and renal toxicities of low and high dose carboplatin in rats. *Proc Am Ass Cancer Res* 1986, **27**, 1669.
23. Bannister SJ, Sternson LA, Repta AJ. Urine analyis of platinum species derived from *cis*-dichlorodiammine platinum (II) by high performance liquid chromatography following derivatization with sodium diethyldithiocarbamate. *J Chromatogr* 1979, **173**, 333–342.