Anti-Cancer Drugs, 4, pp. 85–90

Comparative nephrotoxicity of carboplatin and cisplatin in euvolemic and dehydrated rats

Franck Martinez,[1] Gilbert Deray,[1] Michèle Dubois,[3] Hélène Beaufils,[5] Claude Jacquiaud,[2] Richard Bourbouze,[4] M Benhmida,[1] Marie-Chantal Jaudon[2] and Claude Jacobs[1]

Departments of [1]Nephrology and [2]Biochemistry, and [3]Inserm Ecole de Chirurgie, Hôpital Pitie, Paris, France.
Fax: (33-1) 44-23-99-84
[4]Laboratoire de Chimie Biologique, UER de Biologie Humaine et Expérimentale, Université René Descartes, Paris, France
[5]INSERM U192, Hôpital Necker, Paris, France

The aim of this study was to compare the renal tolerance of cisplatin and carboplatin in euvolemic and dehydrated rats. A total of 79 euvolemic or dehydrated male rats were randomly assigned to receive cisplatin (5 mg/kg body weight, i.p.), carboplatin (40 mg/kg body weight, i.p.) or vehicle. Body weight, serum creatinine, creatinine clearance, fractional excretion of sodium and urinary NAG excretion were recorded on days 1 and 5. Glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and renal histology were determined on day 5. In the euvolemic and dehydrated control and carboplatin groups we observed no change in serum electrolytes, serum creatinine, creatinine clearance, GFR and ERPF. In the euvolemic and dehydrated control groups we observed no change in urinary NAG excretion. Carboplatin induced a slight but significant increase in urinary NAG excretion. In dehydrated rats carboplatin induced a significantly higher increase in urinary NAG excretion than in euvolemic rats. Cisplatin induced a marked and significant decrease in GFR and ERPF, and a significant increase in NAG. Dehydration markedly potentiated cisplatin nephrotoxicity. Euvolemic rats treated with cisplatin exhibited slight renal lesions with a mean score which was similar to the control group. The most extensive lesions were observed in euvolemic and dehydrated cisplatin treated rats with tubular necrosis in the outer stripe of the medulla. The mean total nephrotoxicity score of dehydrated cisplatin treated rats was significantly higher than that of the euvolemic cisplatin treated rats. The results in this study indicate that carboplatin is a promising second generation platinum analog with respect to decreased renal toxicity as compared with cisplatin and may be particularly useful in patients with risk factors such as chronic renal failure or in patients who cannot bear hyperhydration.

Key words: Carboplatin, cisplatin, nephrotoxicity, rat.

Correspondence to G Deray

Introduction

Cisplatin (*cis*-diamminedichloroplatinum II) is a widely used and highly effective antitumor agent against several types of neoplasms including testicular, ovarian and lung tumors, and carcinomas of the head and neck.[1] However the antitumor benefits of cisplatin have been challenged by substantial toxic side effects including nephrotoxicity, gastrointestinal toxicity, neurotoxicity and myelosuppression. Of these there is a general consensus that kidney toxicity is dose limiting, with a reversible, decrease in glomerular filtration rate, in treatment courses which are considered optimal to confer antitumor benefit.[2–4] Various maneuvres, mainly intravenous hydration,[4,5] have been adopted to lessen the incidence of cisplatin nephrotoxicity. Despite these efforts, however, nephrotoxicity has remained dose limiting for cisplatin administration.

Carboplatin (*cis*-diamine-1,1-cyclobutane dicarboxylate platinum II) is a new platinium containing analog of major interest. Early clinical studies established that carboplatin, when administered at the normal phase II dose of 400 mg/m^2, was virtually devoid of nephrotoxicity, ototoxicity and peripheral neurotoxicity.[6–10] In these studies the renal function was measured by monitoring serum creatinine and creatinine clearance. However, the determination of creatinine as a reflection of the glomerular filtration rate has proved to be a relatively insensitive method to monitor cisplatin induced renal damage.[11] Furthermore, whether hydration status modulates carboplatin renal tolerance is not yet known.

Therefore the aim of this study was to compare the renal tolerance of cisplatin and carboplatin in

euvolemic and dehydrated rats with sensitive renal indexes.

Material and methods

Male Sprague–Dawley rats weighing 250–300 g were obtained from Charles River (St Aubin-les-Elbeuf, France). They were conditioned for 5 days prior to the study. Study rats were housed individually in wire bottom stainless steel cages. Food and water were provided *ad libitum*, except as necessary in the groups submitted to dehydration. In these rats access to the water was denied for 24 h before cisplatin, carboplatin or vehicle administration. A total of 79 male rats were each randomly assigned to one of six treatment groups. The treatment groups with their respective test compounds and dosages are outlined in Table 1. Single doses of cisplatin (Bristol Laboratories, Paris, France) or carboplatin (Bristol Laboratories) were administered intraperitoneally on day 1. Rats in the control group received an intraperitoneal injection of saline. The cisplatin dose of 5.0 mg/kg had been previously determined in our laboratory to produce a suitable level of histopathologically evident nephrotoxicity in male Sprague–Dawley rats.[12] The dose of 40 mg/kg was used for carboplatin as it is the maximum non-lethal single dose. Furthermore, since the doses of carboplatin used in human are 4-fold higher than those of cisplatin, an 8-fold increase in carboplatin doses compared with cisplatin allows conclusions as to the clinical relevance of our results.

Body weight, serum creatinine, creatinine clearance, fractional excretion of sodium, electrolytes and urinary NAG excretion were recorded on days 1 and 5 with blood samples being collected from the tail vein.

Table 1. Study design

Group number	Test compound (hydration status)	Dosage (mg/kg)
I ($n = 5$) and VII ($n = 6$)	saline (0.9%) (normohydrated)	—
II ($n = 5$) and VIII ($n = 7$)	saline (0.9%) (dehydrated)	—
III ($n = 5$) and IX ($n = 6$)	cisplatin (normohydrated)	5
IV ($n = 5$) and X ($n = 6$)	cisplatin (dehydrated)	5
V ($n = 10$) and XI ($n = 6$)	carboplatin (normohydrated)	40
VI ($n = 10$) and XII ($n = 8$)	carboplatin (dehydrated)	40

The glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined on day 5 in 39 rats. On day 5, rats were anesthetized with inactin (100 mg/kg, i.p.) and placed on a thermoregulation table. The left carotid artery was cannulated for blood sampling. After tracheostomy, a catheter was inserted into the jugular vein for infusion of inulin (8.3 mg/ml) and PAH (*para*-amino hippurate) (1 mg/ml) in 0.9% NaCl at a rate of 6 ml/h. Before infusion, a 5 ml isotonic saline bolus containing inulin (125 mg) and PAH (5.5 mg) was perfused within 3 min.

After a 1 h equilibrium period, three 30-min urine collections with concurrent arterial blood samples (0.5 ml) were obtained for determination of hematocrit, GFR and ERPF. Inulin and PAH concentrations in plasma and urine were measured with a colorimetric method as previously described.[2] The calculation of fractional excretion of sodium was done by:

$$\% \text{ FE Na} = (U/P)Na/(U/P) \text{ CR} \times 100$$

Creatinine was measured without storage by the Jaffe chromogen reaction. NAG was measured by an enzymatic assay as previously described.[3]

On day 5 all rats were sacrificed, and the kidneys were collected and preserved in 10% neutral buffered formaldehyde for histopathological evaluation. Kidney tissue was embedded in paraffin, sectioned at approximately 3.6 μm and stained with hematoxylin & eosin. Microscopic renal lesions were scored in a blind fashion by our pathologist (H.B.). Lesions encountered in the renal cortex and the outer stripe of the medulla included tubular degeneration, tubular necrosis, tubular dilatation and mononuclear cell infiltration.

The severity of each of these lesion was scored as follows: 0, absence of lesion: 1, mild; 2, moderate; 3, severe. The lesion score was then expressed as the mean of all lesions scores.

Statistical analysis

All data analyses were performed on an IBM PC microcomputer using the Number Cruncher Statistical System (NCSS). Baseline and experimental values in each rat were compared using the non-parametric Wilcoxon test. Comparisons among groups were performed using the non-parametric Neuman–Keuls test. Statistical significance was defined as $p < 0.05$. Results are presented as mean $\pm$ SEM.

Results

Saline injected rats exhibited a 10–19% body weight gain during the course of the study. Body weight remained stable in carboplatin treated rats and significantly decreased in cisplatin treated rats (Table 2).

In the euvolemic and dehydrated controls and carboplatin groups we observed no change in serum electrolytes (data not shown), serum creatinine and creatinine clearance (Table 3). Cisplatin induced a significant increase in serum creatinine from 34 ± 2 to 376 ± 135 μmol/l and a significant decrease in creatinine clearance from 0.97 ± 0.07 to 0.14 ± 0.06 ml/min ($p < 0.05$) (Table 3). These alterations were markedly potentiated in dehydrated cisplatin treated rats (Table 3).

Table 2. Pre- and post-treatment body weights

Group number	Body weight (g)	
	pre-treatment	post-treatment
I Saline (normohydrated)	295 ± 4	329 ± 6[a]
II Saline (dehydrated)	245 ± 5	304 ± 5[a]
III Cisplatin (normohydrated)	288 ± 6	276 ± 12[a]
IV Cisplatin (dehydrated)	262 ± 4	253 ± 4[a]
V Carboplatin (normohydrated)	290 ± 5	300 ± 6
VI Carboplatin (dehydrated)	252 ± 3	268 ± 10

[a] $p < 0.05$ when compared with pre-treatment value.

In the euvolemic and dehydrated control group we observed no change in urinary NAG excretion (Table 4). Carboplatin induced a slight but significant increase in urinary NAG excretion from 107 ± 5 on day 1 to 161 ± 13 mmol/l/mmol creatinine on day 5 ($p < 0.05$) in euvolemic rats (Table 4). In dehydrated rats carboplatin induced a significantly higher increase in urinary NAG excretion than in euvolemic rats (116 ± 14 versus 204 ± 25 mmol/l/mmol creatinine on days 1 and 5, respectively, $p < 0.05$) (Table 4).

Cisplatin induced a significant increase in urinary NAG excretion in euvolemic rats (108 ± 10 versus 295 ± 36 on days 1 and 5, respectively, $p < 0.05$). This effect was markedly potentiated in dehydrated rats. Those modifications were significantly different from those observed in control and carboplatin treated rats (Table 4).

Fractional excretion of sodium did not increase in control rats or carboplatin treated rats (Table 4).

FeNa was significantly elevated in cisplatin treated rats when compared with pretreatment values and control and carboplatin treated rats. Post-treatment FeNa was higher in dehydrated than euvolemic cisplatin treated rats (21.8 ± 7.0 versus 10.2 ± 4.8, $p < 0.05$) (Table 4).

GFR (Figure 1) and ERPF (Figure 2) were 0.69 ± 0.1 and 1.9 ± 0.4 ml/min/100 g, respectively, in euvolemic control rats on day 5. Dehydrated control rats had similar values (GFR: 0.8 ± 0.08 ml/100 g; ERPF: 2.2 ± 0.3 ml/min/100 g).

In euvolemic carboplatin treated rats, GFR and ERPF were 0.8 ± 0.1 ml/min/100 g and 1.9 ± 0.3 ml/min/100 g, respectively; values which were

Table 3. Pre- and post-treatment serum creatinine (SCR) and creatinine clearance (CRCl)

Group number	CRCl (ml/min/100 g)		SCR (μmol/l)	
	pre-treatment	post-treatment	pre-treatment	post-treatment
I Saline (normohydrated)	0.95 ± 0.08	0.90 ± 0.15	34 ± 2	38 ± 2
II Saline (dehydrated)	0.75 ± 0.04	0.85 ± 0.06	38 ± 2	33 ± 2
III Cisplatin (normohydrated)	0.97 ± 0.07	0.14 ± 0.06	34 ± 2	376 ± 135[a,b]
IV Cisplatin (dehydrated)	0.75 ± 0.08	0.011 ± 0.004	34 ± 2	612 ± 71[a]
V Carboplatin (normohydrated)	0.95 ± 0.07	0.91 ± 0.04	33 ± 2	30 ± 0
VI Carboplatin (dehydrated)	0.87 ± 0.06	0.87 ± 0.07	33 ± 2	34 ± 2

[a] $p < 0.05$ when compared with control and CBDCA treated rats and pre-treatment value.
[b] $p < 0.05$ when compared with cisplatin dehydrated rats.

Table 4. Pre- and post-treatment sodium excretion fraction and urinary NAG excretion

Group number	FeNa (%)		NAG (μmol/l/mmol creatinine)	
	pre-treatment	post-treatment	pre-treatment	post-treatment
I Saline (normohydrated)	0.36 + 0.04	0.29 + 0.03	80 ± 6	106 ± 14
II Saline (dehydrated)	0.37 + 0.04	0.30 + 0.02	93 ± 8	109 ± 4
III Cisplatin (normohydrated)	0.40 + 0.03	10.2 ± 4.75[a,b,c]	108 ± 10	295 ± 36[a,b,c]
IV Cisplatin (dehydrated)	0.33 + 0.02	21.8 + 6.95[a,c]	132 ± 7	659 ± 240[a,c]
V Carboplatin (normohydrated)	0.39 + 0.02	0.25 + 0.02	107 ± 5	161 ± 13[c]
VI Carboplatin (dehydrated)	0.37 + 0.02	0.27 + 0.01	116 ± 14	204 ± 25[c,d]

[a] $p < 0.05$ when compared with control and carboplatin treated rats on day 5.
[b] $p < 0.05$ compared with cisplatin dehydrated rats.
[c] $p < 0.05$ compared with pre-treatment value.
[d] $p < 0.05$ compared with euvolemic carboplatin treated rats.

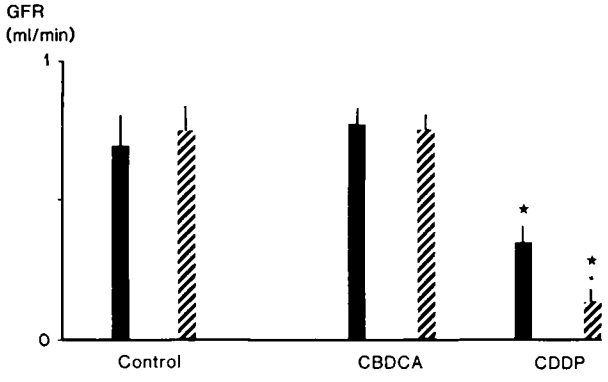

Figure 1. Effects of carboplatin and cisplatin on GFR. $^{*}p < 0.05$ compared with control and carboplatin treated rats. $^{+}p < 0.05$ compared with euvolemic cisplatin treated rats. ■, euvolemic; ▨, dehydrated rats.

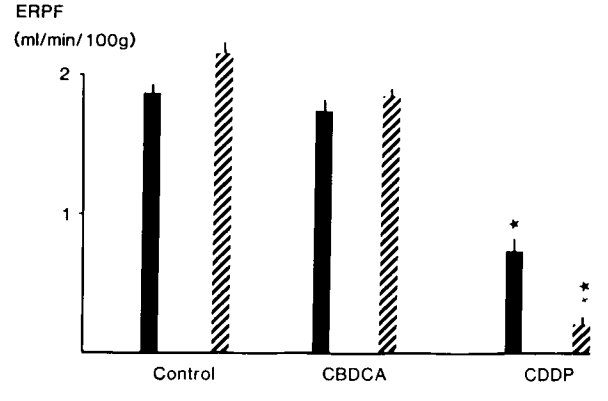

Figure 2. Effects of carboplatin and cisplatin on ERPF. $^{*}p < 0.05$ compared with control and carboplatin treated rats. $^{+}p < 0.05$ compared with euvolemic cisplatin treated rats. ■, euvolemic; ▨, dehydrated rats.

not different from the control group. In dehydrated carboplatin treated rats the renal function remained unaltered (GFR: 0.7 ± 0.08 ml/min/100 g; ERPF: 2.0 ± 0.18 ml/min/100 g) (Figures 1 and 2).

Cisplatin induced a significant decrease in GFR (0.3 ± 0.15 ml/min/100 g) and ERPF (0.8 ± 0.4 ml/min/100 g) (Figures 1 and 2) in euvolemic rats compared with control and carboplatin treated rats ($p < 0.05$).

Dehydration markedly potentiated cisplatin nephrotoxicity, GFR and ERPF being 0.13 ± 0.07 ml/min/100 g and 0.3 ± 0.19 ml/min/100 g, respectively, on day 5 post-treatment ($p < 0.05$ compared with euvolemic cisplatin rats) (Figures 1 and 2).

The mean nephrotoxicity scores for each treatment group are shown by treatment group in Figure 3. The control group exhibited almost no

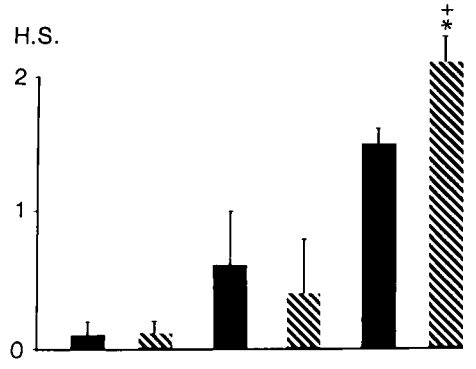

Figure 3. Histological score (H.S.). $^{*}p < 0.05$ compared with control and carboplatin treated rats. $^{+}p < 0.05$ compared with euvolemic cisplatin treated rats. ■, euvolemic; ▨, dehydrated rats.

lesions (0.1 ± 0.1) and provided baseline data against which similar lesions in drug treated rats were compared.

Euvolemic rats treated with cisplatin (group V) exhibited slight tubular degeneration, peritubular mononuclear infiltration, tubular dilatation and tubular fibrosis without tubular necrosis. These lesions were qualitatively and quantitatively similar to those in the control group except for tubular degeneration, which was more frequent in this group (0.6 ± 0.4). The mean total nephrotoxicity score of dehydrated carboplatin (0.4 ± 0.3) treated rats was also similar to that of euvolemic carboplatin rats.

Extensive lesions were observed in euvolemic cisplatin treated rats with tubular necrosis in the outer stripe of the medulla. Tubular necrosis was observed in five rats of this group. The mean score (1.45 ± 0.08) was significantly higher than in control and carboplatin treated rats.

The mean total nephrotoxicity score (2.05 ± 0.06) of dehydrated cisplatin was higher than euvolemic cisplatin treated rats (Figure 3).

Discussion

An important preclinical feature of carboplatin and an essential prerequisite selection criterion was its failure to elicit nephrotoxicity in animals.[14,15] With respect to the preclinical toxicology and phase I clinical trials, carboplatin was also not associated with significant nephrotoxicity.[9,16] However, in those studies the carboplatin renal tolerance was assessed with very poor indexes, such as blood urea nitrogen and serum creatinine.

Renal function is best described on the basis of glomerular and tubular function, renal plasma and blood flow, and urine flow. Glomerular function is classically determined by serum creatinine or creatinine clearance. It must be stressed that the serum creatinine level will not reflect any abnormal renal status until the true GFR has been reduced to less than 50 ml/min/m^2.[11] Furthermore, analysis of creatinine clearance necessitates timed collection of all urinary output over a period of 24 h. Another factor affecting this method includes the patient's own production of creatinine which depends on body muscle mass. Finally, the value of the assay is limited because it measures chromogens and not specifically creatinine. Therefore, there is a need for further evaluation of the renal tolerance of carboplatin with sensitive indexes to evaluate renal function.

Using such indexes of renal hemodynamic and renal tubular damage we have confirmed[12] that cisplatin is nephrotoxic with a marked alteration in renal hemodynamics along with an increase in urinary NAG excretion and tubular necrosis. Furthermore, cisplatin induced a marked increase in FeNa. Physiologically, a prerenal process without tubular dysfunction would be expressed by increased tubular reabsorption of sodium and decreased FeNa whereas tubular injury is associated with increased FeNa.[17] As previously described, the renal tolerance of cisplatin was markedly modified by dehydration. In our model, carboplatin, however, did not produce any significant nephrotoxicity at eight times the cisplatin dose. Furthermore, dehydration did not modify the renal tolerance of carboplatin.

The most serious side effect of cisplatin is nephrotoxicity. After multiple courses of cisplatin, a decrease of about 40% in GFR has been reported.[18] The cisplatin analog carboplatin has not been reported nephrotoxic at conventional dose levels. The reduced protein binding and greater stability of carboplatin in body fluids and therefore increased renal excretion compared with cisplatin are supposed to account for the absence of nephrotoxicity.[19] Also in animal models, carboplatin enhanced nuclear protein phosphorylation in tumor cells more than cisplatin did, but caused much less protein phosphorylation in the normal liver and kidney cells. This suggests some selective toxicity towards tumor cells and may in part explain the decreased nephrotoxicity of carboplatin.[3]

Carboplatin has been recommended as an alternative to cisplatin in patients with impaired renal function. We indeed confirm that the renal tolerance of carboplatin is much better than that of cisplatin.

It has also been suggested that carboplatin can be administered without hydration through intravenous bolus in an outpatient setting and on a monthly schedule.[7] However, the effects of dehydration on carboplatin nephrotoxicity have never been confirmed either in humans or in animals. Our results clearly demonstrate that dehydration does not modify the effects of carboplatin on ERPF, GFR, FeNa and histological lesions, whereas it does increase cisplatin induced nephrotoxicity. However, it must be outlined that urinary NAG excretion was higher in dehydrated than euvolemic carboplatin treated rats. NAG is an enzyme located in the lysosomes of renal tubular cells and an increase in its urinary excretion is a good index of renal tubular damage.[15] Our results

suggest a potential for dehydration to modify carboplatin renal tolerance.

These results may explain why a few cases of acute renal failure induced by carboplatin have been reported in the literature in patients with risk factors such as chronic renal failure and/or dehydration.[9,20,21] We suggest that in such high risk patients hydration should also be used along with carboplatin.

The results of our study indicate that carboplatin is a promising second generation platinium analog with respect to decreased renal toxicity.

References

1. Coehrer PJ, Einhorn LH. Cisplatin. *Ann Intern Med* 1984; **100**: 704.
2. Galli A. Dosage colorimétrique de l'inuline et du PAH dans le sang et les urines. Applications de l'analyse automatique. *Ann Biol Clin* 1965; **23**: 931.
3. Harrap KR, Jones M, Wilkinson CR, *et al.* Antitumor, 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.* London: Academic Press.
4. Orols RF, Corden BJ, Jacobs J, *et al.* High dose cisplatin in hypertonic saline. *Ann Intern Med* 1984; **100**: 19.
5. Stark JJ, Howell SB. Nephrotoxicity of *cis*-platinium (II) dichlorodiammine. *Clin Pharmacol Ther* 1978; **23**: 461.
6. Adams M, Kerby IJ, Rocker I, *et al.* A comparison of the toxicity and efficacy of cisplatin and carboplatin in advanced ovarian cancer. The Swons Gynaecological Cancer Group. *Acta Oncol* 1989; **28**: 57.
7. Calvert AH, Urie J. The cost of carboplatin treatment. *Sem Oncol* 1991; **18**: 28.
8. Kreisman H, Goutson M, Modeas C, *et al.* Cisplatin–carboplatin therapy in extensive non-small cell lung cancer: a cancer and leukemia groups study. *Eur J Cancer* 1990; **26**: 1057.
9. Levine BS, Henry MC, Port CD, *et al.* Nephrotoxic potential of *cis*-diammine-dichloroplatinum and four analogs in male fisher 344 rats. *J Natl Cancer Inst* 1981; **67**: 201.
10. Shea TC, Flaherty M, Elias A, *et al.* A phase I clinical and pharmacokinetic study of carboplatin and autologous bone marrow support. *J Clin Oncol* 1989; **7**: 651.
11. Becker JA. Evaluation of renal function. *Radiology*, 1991; **179**: 337.
12. Deconti RC, Toftness RC, Lange RC, *et al.* Clinical and pharmacological studies with *cis*-diammine dichloroplatinium (II). *Cancer Res* 1973; **33**: 1310.
13. Bourbouze R, Gluckman JC, Frantz P, *et al.* Early monitoring of human renal transplantation by *N*-acetyl-β-D-glucosaminidase enzyme activities in urine. *Clin Chem Acta*, 1985; **149**: 185.
14. Bregman CL, Williams PD. Comparative nephrotoxicity of carboplatin and cisplatin in combination with tobramycin. *Cancer Chemother Pharmacol* 1986; **18**: 117.
15. Le Hir M, Dubach UC, Schmidt U. Quantitative distribution of lysosomal hydrolases in the rat nephron. *Histochemistry* 1979; **63**: 245.
16. 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.
17. Espinel CH. The FeNa test: use in the differential diagnosis of acute renal failure. *J Am Med Ass* 1976; **236**: 579.
18. Fjeloborg P, Sorensen J, Helkjaer PE. The longterm effect of cisplatin on renal function. *Cancer* 1986; **58**: 2214.
19. Goldstein RS, Mayor GH. The nephrotoxicity of cisplatin. *Life Sci* 1983; **32**: 685.
20. Deray G, Ben-Othman T, Brillet G, *et al.* Carboplatin induced acute renal failure. *Am J Nephrol* 1990; **10**: 431.
21. Reed E, Jacobs J. Carboplatin and renal dysfunction. *Ann Intern Med* 1989; **110**: 409.
22. Curt GA, Grygiel JJ, Corden BJ, *et al.* A phase I and pharmacokinetic study of diammine cyclobutane dicarboxylate platinum (NSC 241240). *Cancer Res*, 1983; **43**: 4470.
23. Deray G, Dubois M, Beaufils H, *et al.* Effects of nifedipine on cisplatinum induced nephrotoxicity. *Clin Nephrol* 1988; **3**: 146.
24. Harland SJ, Newell DR, Siddik ZH, *et al.* Pharmacokinetics of *cis*-diammine-1,1-cyclobutane dicarboxylate platinum (II) in patients with normal and impaired renal function. *Cancer Res* 1984; **44**: 1693.
25. Krakoff IH. Nephrotoxicity of *cis*-dichlorodiammine platinum (II). *Cancer Treat Rep* 1979; **63**: 1523.
26. Litterst CL. Alterations in the toxicity of *cis*-dichlorodiamine platinium II and in tissue localization of platinium as a function of NaCl concentration in the vehicle of administration. *Toxicol Appl Pharmacol* 1981; **61**: 99.
27. Lovett D, Kelsen D, Eisenberger M, *et al.* A phase II trial of carboplatin and vinblastin in the treatment of advanced squamous cell carcinoma of the esophagus. *Cancer* 1991; **67**: 354.
28. Volling P, Staar S, Achterrath W, *et al.* Carboplatin plus radiation therapy in head and neck cancer. *Sem Oncol* 1991; **18**: 17.

(Received 10 November 1992; accepted 2 December 1992)