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Urinary protein and enzyme excretion in patients receiving chemotherapy with the *cis*-platinum analogs carboplatin (CBDCA, JM8) and iproplatin (CHIP, JM9)

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Summary. Urinary protein and enzyme excretion was measured in 33 patients with solid tumours receiving chemotherapy with the *cis*-platinum analogs carboplatin (JM8, CBDCA) and iproplatin (JM9, CHIP). The patients were given up to six courses of the drugs at 4-week intervals, and serial urine samples were collected weekly for periods up to 28 weeks. Overall there was no significant increase in the alkaline phosphatase (ALP), lactate dehydrogenase (LD), and *N*-acetyl glucosaminidase (NAG) excretion of the first posttreatment samples compared with the pretreatment samples. During the course of treatment there were transient increases in all three enzymes, some quite marked. There was no consistent increase in urinary protein or enzyme excretion during the period of treatment, suggesting that there was no cumulative nephrotoxicity. There was no change in creatinine clearance or urinary β_2-microglobulin content. Iproplatin appeared marginally more toxic on the basis of elevated NAG and ALP during the second half of the treatment periods compared with the first ($P < 0.01$ and < 0.025, respectively).

Introduction

Cis-Dichlorodiammine platinum (II) (CDDP, cisplatin) is a useful chemotherapeutic agent of proven value against ovarian and testicular tumours [17, 18]. Nephrotoxicity has limited the use of this agent [12, 20, 28, 30] but with the introduction of hydration and forced diuresis, the incidence of renal toxicity has markedly reduced [8, 15, 26] or can be at least partly reversible [8]. Nevertheless there is some long-term impairment [10]. As a result, there has been great interest in developing new platinum analogs with similar antitumour activity as the parent drug but without the attendant nephrotoxicity. Two new second generation platinum analogs, diamine-1, 1-cyclobutane dicarboxylate Pt II, carboplatin (CBDCA, JM8) and *cis*-dichloro-*trans*-dihydroxy- bis-isopropylamine Pt IV, iproplatin (CHIP, JM9) with proven anti-cancer activity in animals and little renal toxicity [23, 27] are presently being evaluated in phase II clinical trials.

The dose-limiting toxicity in a phase I assessment was myelosuppression [5]. Although there was virtually no evidence of nephrotoxicity as determined by routine measurements of serum urea and creatinine concentrations and the creatinine clearance [5] these tests are relatively insensitive. It is recognised that a better index of renal tubular damage would be the excretion of urinary enzymes [3, 9, 19, 21].

In the present study 33 patients with solid tumours were treated with either carboplatin or iproplatin and we monitored any changes in renal function during therapy by estimating the creatinine clearance. The urinary excretion of β_2-microglobulin, protein, N-acetyl B-glucosaminidase (NAG), alkaline phosphatase (ALP) and lactate dehydrogenase (LD) was measured as an index of nephrotoxicity.

Materials and methods

Patients and sample collection procedure

Serial random urine samples were collected at weekly intervals from a group of 33 patients, aged 29-75y, 20 males, 13 females, with histologically proven solid tumours 23 of whom were being treated with carboplatin and 10 with iproplatin. The patients in each group are identified in Table 1. Most had advanced disease and had already received treatment with other chemotherapeutic agents or radiotherapy. The first urine sample was collected immediately before starting chemotherapy and on subsequent visits to the clinic prior to receiving treatment. Each urine sample (20 ml) was added to a Universal bottle containing 1 ml 2M Tris-HCl buffer pH 7.8 containing 60 mM sodium azide and 0.6 mM chlorhexidine. The samples were usually kept at 4° C until analysed and stored thereafter at −20° C.

Chemotherapy

The drugs obtained from Bristol-Myers Pharmaceuticals, Slough SL3 6EB were dissolved in 5 percent dectrose-saline (250 ml) and administered intravenously over thirty minutes. All patients, with the exception of ovarian tumour patients, were entered into a multicentre randomised trial comparing carboplatin with iproplatin and hence there was no selection of patients for the two drugs. Patients who had ovarian tumours were given carboplatin only. The starting dose for carboplatin was 400 mg/m^2 which was given at four weekly intervals. Patients with prior treatment (extensive radiation treatment or more than six months of previous myelo-suppressive therapy) were

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Table 1. Clinical details of patients treated with the platinum analogs JM8 or JM9

Diagnosis	No of patients treated with	
	JM8	JM9
Ovarian cancer	8	–
Mesothelioma	5	4
Small cell carcinoma of lung	4	1
Gastric carcinoma	1	1
Malignant melanoma	1	1
Bronchogenic carcinoma	1	–
Retroperitoneal sarcoma	1	–
Embryonal rhabdamyosarcoma	1	–
Non-Hodgkin's lymphoma	1	–
Renal carcinoma	–	1
Testicular carcinoma	–	1
Synovial sarcoma	–	1
Total	23	10

started on 360 mg/m^2. The starting dose for iproplatin was 300 mg/m^2 and patients with prior treatment were given 90 percent dose level (270 mg/m^2) at four weekly intervals. The patients received between one and six courses of the drugs.

Techniques

The techniques used for analysis of the urine samples were as follows:

Dialysis. The urine samples were dialyzed overnight against 50 volumes 10 m*M* TRIS-HCl buffer (pH 7.8) containing 3 m*M* sodium azide. After the buffer was changed, dialysis was continued for a further 3 h.

Protein [1]. 500 µl urine was added to 3 ml solution comprising 50 mg Coomassie blue B-G250 in 0.4 *M* ethanol containing 0.15 *M* orthophosphoric acid. The mixture was incubated at 37° C for 15 min, and the absorbance at 600 nm measured.

Creatinine [16]. 25 µl urine was added to 3 ml distilled water, followed by 1 ml 50 m*M* picric acid and 0.5 ml 1.4 *M* sodium hydroxide. After 30 min at room temperature the absorbance at 500 nm was measured.

Lactate dehydrogenase [29]. 500 µl dialyzed urine was added to 500 µl 0.1 *M* sodium phosphate buffer containing 0.34 m*M* NADH in the measuring cuvette of an LKB Reaction Rate Analyser (LKB, Bromma, Sweden). After 15 min incubation at 37° C, 100 µl 0.55 m*M* sodium pyruvate was added with mixing, and the rate of change in absorbance at 340 nm measured.

N-acetyl-β-glucosaminidase [22]. 100 µl dialyzed urine was added to 500 µl 5 m*M* *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide in 0.1 *M* sodium citrate buffer (pH 4.5). The mixture was incubated for 2 h at 37° C and the reaction then stopped by the addition of 2.5 ml 0.6 *M* sodium bicarbonate buffer (pH 10.0), after which the absorbance at 410 nm was measured. Blanks were prepared by incubating the buffered substrate for 2 h without urine, adding 2.5 ml bicarbonate buffer and 100 µl urine, and then measuring the absorbance at 410 nm.

Alkaline phosphatase [2]. 500 µl dialyzed urine was added to 500 µl 0.2 *M* 2-amino-2-methyl propanol buffer (pH 10.5) containing 0.1 m*M* magnesium chloride and 1 µ*M* zinc chloride. The reaction was intiated by the addition of 100 µl 0.1 *M* *p*-nitrophenyl phosphate; after 2 h incubation at 37° C the reaction was stopped by the addition of 2 ml 0.2 *M* sodium hydroxide, and the absorbance measured at 410 nm. Blanks were prepared by incubating the buffered substrate without urine for 2 h and then adding 2.5 ml sodium hydroxide and 500 µl urine.

β_2-Microglobulin. Urinary β_2-microglobulin was measured by a radioimmunoassay procedure using the Phadebas kit (Pharmacia Ltd, Milton Keynes, UK).

The total protein, β_2-microglobulin concentration, and enzyme activities of the urine samples were expressed in g, µg, and IU per gram creatinine, respectively.

Chemicals

NADH, TRIS, and sodium pyruvate were obtained from BCL Lewes (UK), *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide from Sigma (Poole, UK), and all other chemicals from BDH (Poole, UK).

Results

The results of the analyses of urinary enzyme and protein excretion of the patients treated with carboplatin or iproplatin are shown in Figs. 1–4. For each analyte the mean, median, and range of values obtained are illustrated starting with the pretreatment sample (week 0) and followed by the posttreatment samples collected at weekly intervals. Reference ranges were obtained by analysis of samples from 79 healthy volunteers, one third of whom were males. The mean, median, and upper limit of the reference range were for protein, 59, 44, and 173 mg/g creatinine; for ALP, 0.7, 0.4, and 2.2 IU/g creatinine, for NAG, 1.5, 1.1, and 5.0 IU/g creatinine; and for LD, 4.5, 2.5, and 14 IU/g creatinine. Any increases in urinary enzyme excretion were identified as sporadic if they were not maintained in at least two successive weekly samples and persistent if the abnormality continued.

In no patient did the β_2-microglobulin excretion exceed the upper limit of the reference range (0.5 µg/l). Creatinine clearance was measured at 4-week intervals, the mean and range for those given carboplatin being 77 ± 26 ml/min and for those given iproplatin, 100 ± 27 ml/min (Table 2). Six of those given carboplatin but none of those given iproplatin had creatinine clearance less than 50 ml/min. In no patient were there significant reductions in creatinine clearance during treatment.

The changes in urine protein and enzyme excretion in the two groups of patients are summarized in Table 3. Abnormal amounts of at least one of the four analytes were found pretreatment in seven (30%) of those given carboplatin and four (40%) of those given iproplatin. During treatment, 13 (56%) of those given carboplatin and two (20%) of those given iproplatin showed sporadic increases in urinary protein. One patient in each group had markedly increased urinary protein both pre- and posttreatment, but normal creatinine clearance.

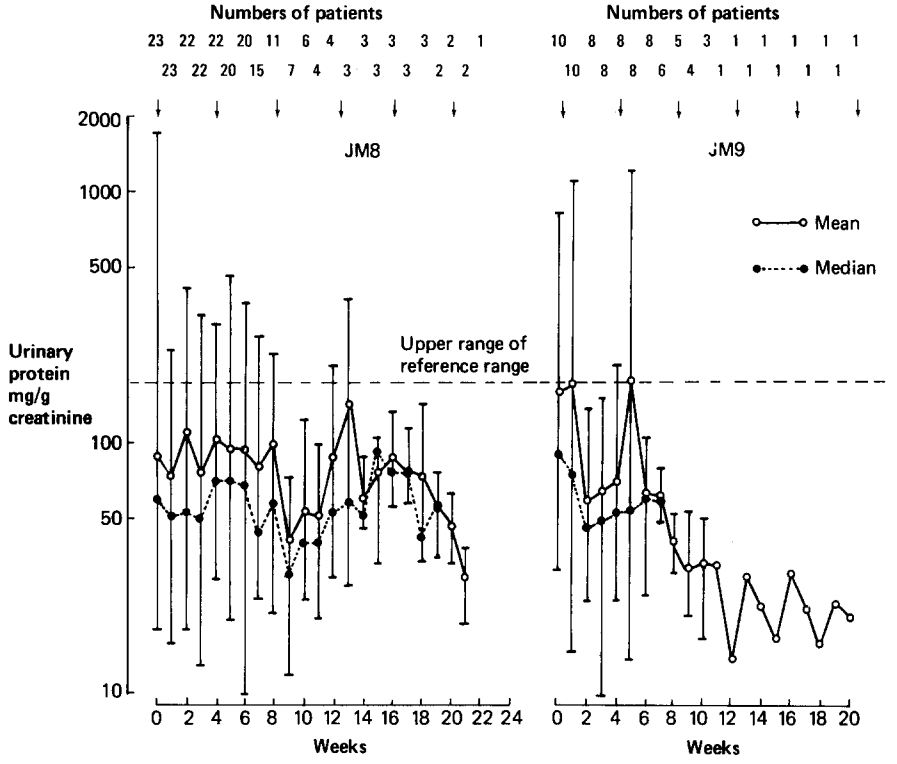

Fig. 1. Urinary protein excretion in patients receiving JM8 or JM9

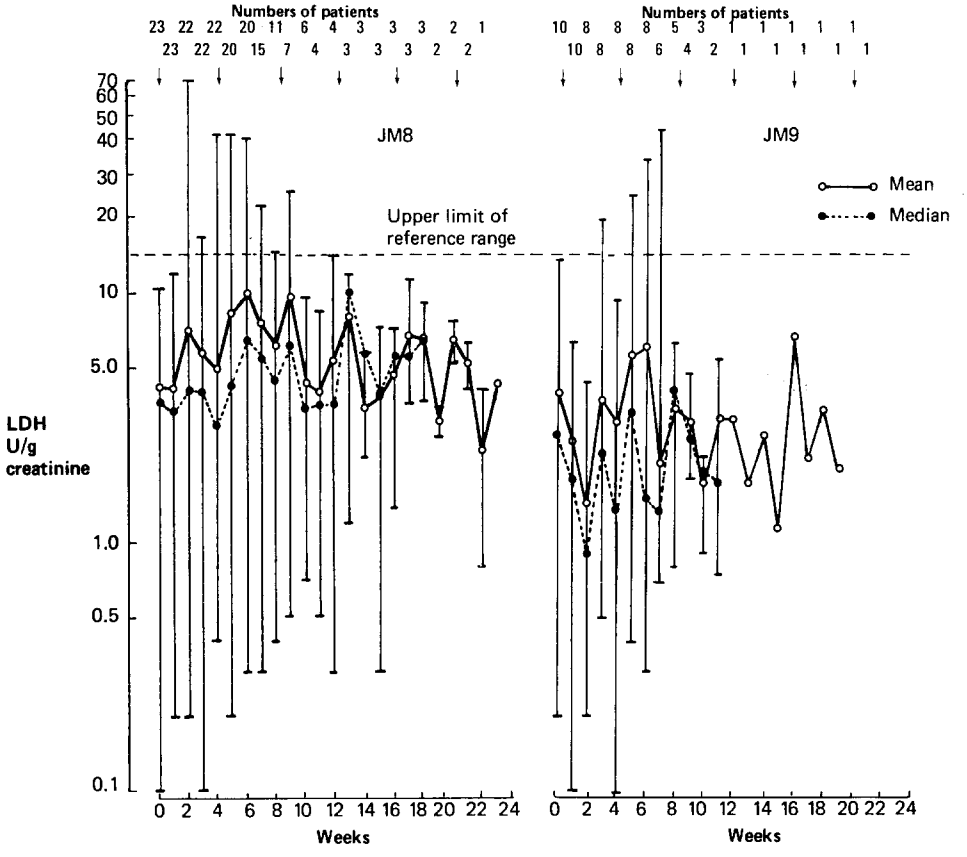

Fig. 2. Urinary LDH excretion in patients receiving JM8 or JM9

Table 2. Creatinine clearances in patients with solid tumours during treatment with JM8 (CBDCA) or JM9 (CHIP)

Time (weeks)	Number of cases	Creatinine clearances ml/min	
		Range	Mean ± SD
0 (Pre-treatment	33	26.3–149.4	84.6±29
4	29	21.3–159	93.5±29
8	25	38.1–123	85.0±34
12	18	33.9–146	86.7±35
16	11	52.2–201	87.3±41
20	8	59.0–140	90.6±29
24	6	54.4–132	88.7±27

Increased urinary ALP was found pretreatment in five (22%) of those given carboplatin and one (10%) of those given iproplatin. During treatment, 15 (65%) of those given carboplatin and three (30%) of those given iproplatin showed increases in urinary ALP. Three (26%) patients given carboplatin and one (10%) given iproplatin showed consistent increases. The three patients given carboplatin had poor creatinine clearance (<45 ml/min), but with the patient given iproplatin creatinine clearance was good (>110 ml/min). Sporadic increases in ALP activity were found in 11 (47%) of those given carboplatin and two (20%) of those given iproplatin.

Increased urinary NAG was found pretreatment in five (22%) of those given iproplatin and one (10%) of those given carboplatin. During treatment, nine (39%) of those given carboplatin and four (40%) of those given iproplatin showed increases in urinary NAG. Three (13%) of those given carboplatin and one (10%) given iproplatin showed consistent increases; of the latter four patients, creatinine clearance was less than 40 ml/min in two given carboplatin but greater than 100 ml/min in the other patients. Sporadic increases in NAG were found in eight (35%) of those given carboplatin and three (30%) of those given iproplatin.

Increased urinary LD was not found pretreatment in either group, but 11 (48%) of those given carboplatin and three (30%) of those given iproplatin showed increased urinary LD activity. Three (15%) of those given carboplatin showed consistent increases accompanied by poor creatinine clearance. Sporadic increases in LD were found in eight (35%) of those given carboplatin and two those given iproplatin.

Of the patients given carboplatin, two showed consistent increases in both ALP and NAG and one also showed consistent increases in LD and protein, both patients having creatinine clearance <45 ml/min. One patient given iproplatin showed consistent increases in all four analytes but creatinine clearance >110 ml/min. Of the sporadic changes in urinary enzymes, these occurred simultaneously in one patient.

The marked increases in the mean urinary ALP and NAG activities of patients given iproplatin were due to the results of a single patient who showed ALP and NAG activities at least five times the upper limit of the reference range. The same patient also showed at week 3 urinary LD activity nearly three times the upper limit of the reference range, but this fell to well within the reference range at week 5, at which time proteinuria had increased to eight times the upper limit found in the healthy.

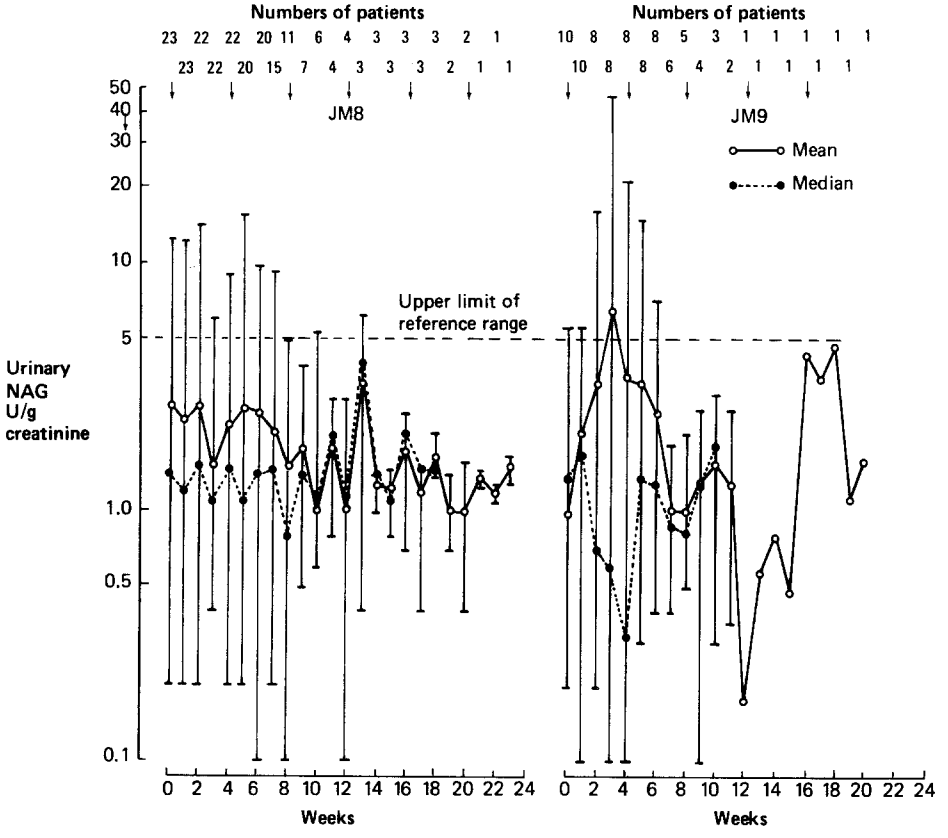

Fig. 3. Urinary NAG excretion in patients receiving JM8 or JM9

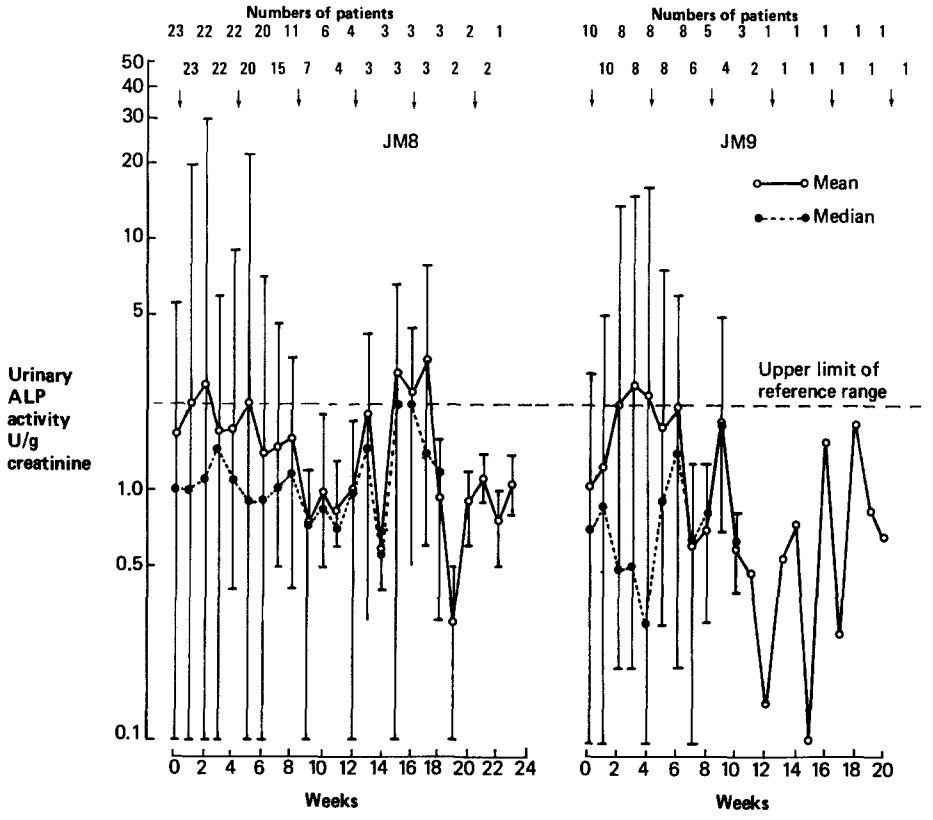

Fig. 4. Urinary ALP excretion in patients receiving JM8 or JM9

Table 3. Urinary enzyme and protein excretion in patients receiving JM8 or JM9

		Number of patients showing			
		No elevation	Pre-treatment elevation	Post-treatment elevation	
				Consistent	Isolated or sporadic
Protein	JM8	10	3	0	11
	JM9	7	1	0	2
ALP	JM8	8	5	4	11
	JM9	5	1	1	2
NAG	JM8	10	5	3	6
	JM9	6	1	2	3
LD	JM8	12	0	3	8
	JM9	7	0	0	3

In one patient receiving carboplatin, urinary NAG activity reached eight times the upper limit of the reference range, with all the other parameters well within their respective reference ranges. One other patient receiving carboplatin showed abnormal levels of all four analytes.

Further statistical analysis of the results was carried out as follows. The results from the pretreatment and first posttreatment samples from each group were compared by the Wilcoxon matched-pairs signed rank test, and the results of samples collected at weeks 0, 4, 8, 12, and 16 were compared by the Mann-Whitney test. In no case were significant differences detected. The results of samples collected from the patients were then divided into two groups, A and B, group A including the pretreatment sample and those collected during the first half of the treatment period, and group B comprising those collected during the second half. The means of the group A samples and the group B samples for the patients given carboplatin or iproplatin were compared by the Wilcoxon test. In those given carboplatin, there was no significant difference in the urinary excretion of protein or any of the enzymes. However, in those given iproplatin, both urinary NAG ($P < 0.01$) and urinary ALP ($P < 0.025$) showed significant increases, but urinary LD and protein did not.

Discussion

Chemotherapy with cisplatin (*cis*-dichlorodiammine platinum II) may be accompanied by renal tubular damage unless there is adequate hydration and diuresis. Urinary enzyme excretion has been recommended as a sensitive indicator of such nephrotoxicity and has been applied in studies with other platinum compounds used as antineoplastic agents [6]. Cisplatin therapy may result in long-term permanent reduction of the glomerular filtration rate, although long-term tubular defects have not been detected [10].

In the first 5 h after cisplatin therapy, de Gislain et al. [7] have found a rise in urinary β_2-microglobulin and a fall in creatinine clearance. There was no relationship of later cumulative nephrotoxicity to fall in β_2-microglobulin, hence creatinine clearance and tubular function clearly need to be separately assessed for new analogs. Goren et al. [13] have found that tubular damage associated with cisplatin nephrotoxicity was cumulative over a 6-month period in 12 children assessed by NAG excretion. The cumulative toxicity with carboplatin and iproplatin has not been

reported. In one study, the urinary excretion of leucine aminopeptidase, NAG, and β_2-glucuronidase has been found to be increased 8 h after treatment with iproplatin [5]. These changes were sufficiently transient to suggest that there was no biochemical evidence of structural damage of the renal tubules.

In a study of carboplatin, iproplatin, and cisplatin, Goren et al. [14] have found that transient rises in NAG, alanine aminopeptidase, and total urinary protein occurred up to a week after cisplatin, but the effects were much less after iproplatin, with no significant change after carboplatin. However, only the first course of therapy was assessed and carboplatin doses were only 300 mg/m^2. Similarly, Pendyala et al. [24] have evaluated changes after one course of iproplatin or cisplatin, and have measured only up to 8 h posttreatment. After iproplatin, NAG and β-glucuronidase rose significantly, but leucine aminopeptidase (LAP) did not. The dose range of iproplatin was lower than in our study. Similarly, Calvert et al. [4] have found no increase in urine enzymes 8 h after 520 mg/m^2 carboplatin in three patients, and Wiltshaw [31] has described carboplatin's lack of nephrotoxicity.

The present investigation was concerned with the possible long-term effects of such drugs on the kidneys during repeated courses. Previous studies have not assessed randomized patients, or such high levels of carboplatin or iproplatin, and have not used LDH, ALP, or β_2-microglobulin to assess renal tubular function. In individual patients, there was no clear evidence of tubular damage, as there was no increase in β_2-microglobulin excretion and the protein and enzyme excretion showed no consistent increases during the treatment program.

The lesions found in patients with cisplatin-induced nephrotoxicity are largely tubular; some workers have identified proximal tubular necrosis as the most common finding, whereas others have found that the distal tubular and collecting duct show the greatest changes [2]. All three enzymes studied are present in high concentration in tubular cells, the ALP being located on the brush border, the NAG in the lysosomes, and the LD in the cytoplasm. Of the three, LD is most likely to be elevated in non-renal disease, being increased in urinary tract infections or when there are bladder lesions [11], and whenever there are substantial losses of protein with the urine. Increased β_2-microglobulin excretion has commonly been taken as an indicator of tubular damage [25]. As significant increases in urinary β_2-microglobulin excretion were not found in these patients, this suggests that any tubular damage is insufficient to have a significant effect on the resorptive capacity of the tubule. Nevertheless, the enzyme changes, particularly those of LD and NAG, are indicative of cell damage but are not clinically significant. This long-term prospective study shows that there is no evidence that any cumulative renal damage occurs after 6 months of treatment with carboplatin.

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