Cancer Chemother Pharmacol (1988) 21: 163–167

Cancer Chemotherapy and Pharmacology

Effects of cisplatin on different measures of glomerular function in the human kidney with special emphasis on high-dose

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Summary. To investigate the effect of high-dose cisplatin (40 mg/m^2 daily for 5 days), ^{51}Cr-EDTA clearance was used as a measure of glomerular filtration rate (GFR). ^{51}Cr-EDTA clearance decreased significantly from 109±3 ml/min * 1.73 m^2 to 68±3 ml/min * 1.73 m^2 after three cycles of cisplatin and remained at this decreased level during the observation period (24 months). To determine the reliability of creatinine as a measure of GFR, we compared the simultaneous clearance of creatinine to that of ^{51}Cr-EDTA. A good correlation between ^{51}Cr-EDTA clearance and creatinine clearance was observed before and 3 months after termination of treatment, but no correlation was found during treatment. S-creatinine decreased significantly during treatment, probably due to muscle wasting. We conclude that s-creatinine and creatinine clearance are unsuitable measures of glomerular function during high-dose cisplatin treatment. All patients developed proteinuria during treatment. The changes in clearance ratios of beta-2-microglobulin/albumin and IgG/albumin show that the proteinuria observed during cisplatin infusion is predominantly of tubular origin, whereas the proteinuria between the treatment periods is mainly of glomerular origin.

Introduction

Cis-diamminedichloroplatinum(II) (cisplatin) has been proven very effective in the treatment of patients with germ cell tumors [4, 21].

Several groups [5, 9, 13, 20] have investigated the acute and long-term nephrotoxicity after "low" doses of cisplatin (20 mg/m^2 body surface area daily for 5 days), and a decrease of 12.5%–23% in glomerular filtration rate (GFR) has been found. In 1984 Ozols et al. [21] stated that high-dose cisplatin (40 mg/m^2 daily for 5 days) could be given without any increase in s-creatinine or decrease in creatinine clearance by using vigorous saline hydration and 3% saline as the vehicle for drug delivery. This has been confirmed in another study using s-creatinine as a measure of kidney function [28].

The protective effect of hypertonic saline given in combination with cisplatin was first described in a study on rats by Litterst [18]. Others have also suggested an improvement in the therapeutic index of cisplatin by increased urinary chloride excretion [3, 8].

The aim of the present study was, first, to investigate if s-creatinine and/or creatinine clearance truly monitor sudden changes in glomerular function in this group of patients. Therefore, these variables were compared with the GFR as measured simultaneously by ^{51}Cr-EDTA clearance during treatment. Second, the nephrotoxicity observed after a high-dose cisplatin regimen was compared to that of a low-dose regimen by the changes in ^{51}Cr-EDTA clearance in the two groups after the same cumulative doses. Third, in order to localize the site of action of cisplatin responsible for the proteinuria induced by high-dose cisplatin, clearance ratios of beta-2-microglobulin/albumin and IgG/albumin were measured during the treatment period.

Materials and methods

Thirty patients with poor prognosis, germ cell tumors [4] were treated with a three-drug combination chemotherapy regimen consisting of 40 mg/m^2 cisplatin and 200 mg/m^2 VP-16 (etoposide) days 1–5 every 3 weeks and 15 mg/m^2 bleomycin every week. The patients were extensively hydrated throughout the 5 days of platinum treatment, receiving 200 ml isotonic saline/h. The cisplatin was mixed in 250 ml 3% sodium chloride and was given over 30 min, followed by 500 ml 20% mannitol. At least three courses of this combination were given to all patients.

The median age of the patients was 35 years (range, 20–52 years). The Median cumulative dose of cisplatin was 1200 mg (range, 800–1200 mg). None of the patients had a previous history of kidney disease, and all initially had normal blood pressure (140–100/95–70 mmHg).

The decrease in GFR in the high-dose cisplatin group was compared with the decrease observed in 41 patients treated with low-dose cisplatin (20 mg/m^2 daily for 5 days, combined with 100 mg/m^2 VP-16 daily for 5 days and 15 mg/m^2 bleomycin every week). These patients were hydrated with 175 ml isotonic saline/h. Cisplatin was mixed with isotonic saline and the median cumulative dose was 800 mg (680–840 mg). The median age for this group was 31 years (19–58 years), and all had a normal ^{51}Cr-EDTA clearance (78–130 ml/min * 1.73 m^2) and normal blood pressure before chemotherapy. Renal functional variables were followed during treatment as stated in Table 1.

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Table 1. Measurements performed in each treatment cycle

Day	0	1				5	6	8	9	11	16	No. of patients
Cisplatin administration		×	×	×	×	×						30
S-creatinine	×						×	×	×	×	×	30
S-urea	×								×			30
U/P albumin	×						×		×		×	8
U/P beta-2-microglobulin	×						×		×		×	6
U/P IgG	×						×		×		×	8
Creatinine clearance	×								×			19
^{51}Cr-EDTA clearance	×								×			30

U/P = urine-to-plasma ratio

GFR was measured by ^{51}Cr-EDTA clearance as described by Groth and Aasted [12]. Creatinine clearance was determined as the ratio between urinary creatinine excretion rate and plasma creatinine concentration. Creatinine in urine and plasma was measured by autoanalyzer as total chromogens by the Jaffe reaction [16].

Fractional clearances of beta-2-microglobulin (b-2-m) (mol. wt. 11,800)/albumin (mol. wt. 69,000) and IgG (mol. wt. 150,000)/albumin were used for determining disorders in renal handling of plasma proteins. The fractional clearances were estimated as: Cprot/Calbumin = (U:P)prot/(U:P) albumin, where U:P refers to the urine-to-midpoint-serum-concentration ratios of b-2-m, albumin, and IgG, and prot refers to b-2-m or IgG. Urine was collected in 24-h periods, and urine and serum samples were stored at −20° C until analyzed.

Urine samples for b-2-m were collected in bottles containing TRIS-buffer solution (Sigma, St. Louis, USA) to adjust urine pH at 6–7. The total quantity of b-2-m in urine and serum was measured using the enzyme-linked immunosorbent assay [1]. Albumin in urine and IgG in urine and serum were measured by single radial immunodiffusion [19]. Rabbit antisera for this were obtained commercially (Dakopatts A 001 and A 090). Serum albumin was determined on the SMA-12/30 using bromocresol green [7].

Statistics. Student's *t*-test for paired data and an analysis of variance were used for statistical analyses. Bonferroni's correction method for multiple comparison [29] and the 0.05 level of significance were used. Values are expressed as mean ± SEM.

Results

Figure 1 shows the changes in ^{51}Cr-EDTA clearance in percent of pretreatment values. GFR suffered progressively during each course of treatment, and after three cycles the average values had decreased significantly from 109±3 ml/min * 1.73 m^2 to 68±3 ml/min * 1.73 m^2. At the end of treatment, more than one-third of the patients had a decrease of 50% or more in ^{51}Cr-EDTA clearance. No significant differences were observed between the value of ^{51}Cr-EDTA clearance obtained just after termination of treatment and the values obtained 3 months after treatment (70±3 ml/min * 1.73 m^2). From 3 to 12 months after termination of treatment, a small but significant increase in ^{51}Cr-EDTA clearance was recorded (70±3–76±3 ml/min * 1.73 m^2).

Although s-creatinine increased during the first cycle of chemotherapy (from 92±3 µmol/l to 99±5 µmol/l), it did not show a pattern corresponding to a steadily decreasing renal function (Fig. 1). Thus, before the second and third cycles, a significant *decrease* in s-creatinine was observed compared to the pretreatment value. In contrast, 3 months after termination of treatment, a significant increase was observed in s-creatinine, which remained at this significantly increased level during the observation period (24 months).

Figure 2 shows the inadequacy of s-creatinine to characterize the actual functional stage in the patients during the process of glomerular deterioration. For 96% of the patients with a moderately decreased ^{51}Cr-EDTA clearance (58–78 ml/min * 1.73 m^2), s-creatinine was normal. This was also the case in 68% of those with considerably decreased ^{51}Cr-EDTA clearance (34–57 ml/min * 1.73 m^2).

During the full treatment period, when ^{51}Cr-EDTA clearance decreased steadily, creatinine clearance remained unchanged (107±6 ml/min before chemotherapy and 94±4 ml/min after three cycles). Thus, the correlation

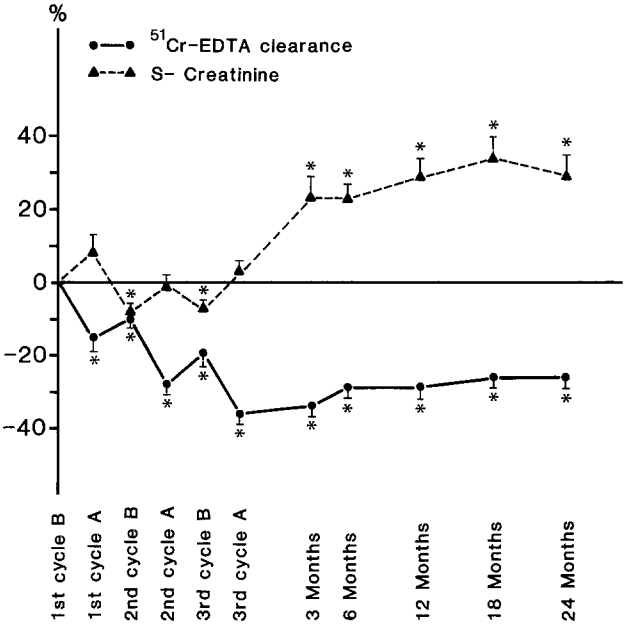

Fig. 1. Percent changes in ^{51}Cr-EDTA clearance and s-creatinine compared to pretreatment values during and after termination of treatment. *B*, before treatment cycle (day 0); *A*, after treatment cycle (day 9); *, $P < 0.05$ compared to pretreatment value

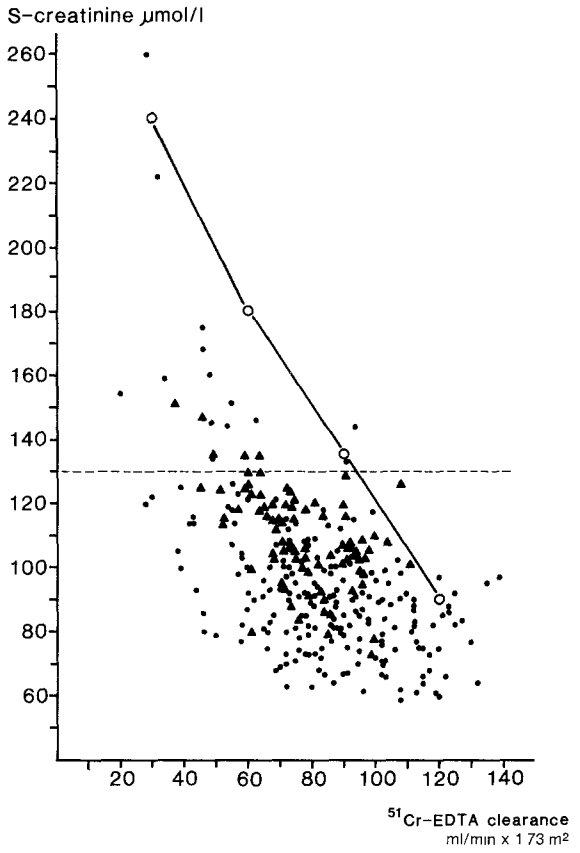

Fig. 2. S-creatinine levels vs. ^{51}Cr-EDTA clearance in 30 patients. The line (○ ——— ○) represents the hypothetical relationship between glomerular filtration rate and s-creatinine, assuming that creatinine is excreted solely by glomerular filtration. In this hypothetical case, the initial s-creatinine = 90 µmol/l and ^{51}Cr-EDTA clearance = 120 ml/min * 1.73 m^2, respectively. The broken line (------) represents the upper limit of normal s-creatinine in our laboratory (130 µmol/l). ● represents values obtained during treatment and ▲ values after termination of treatment

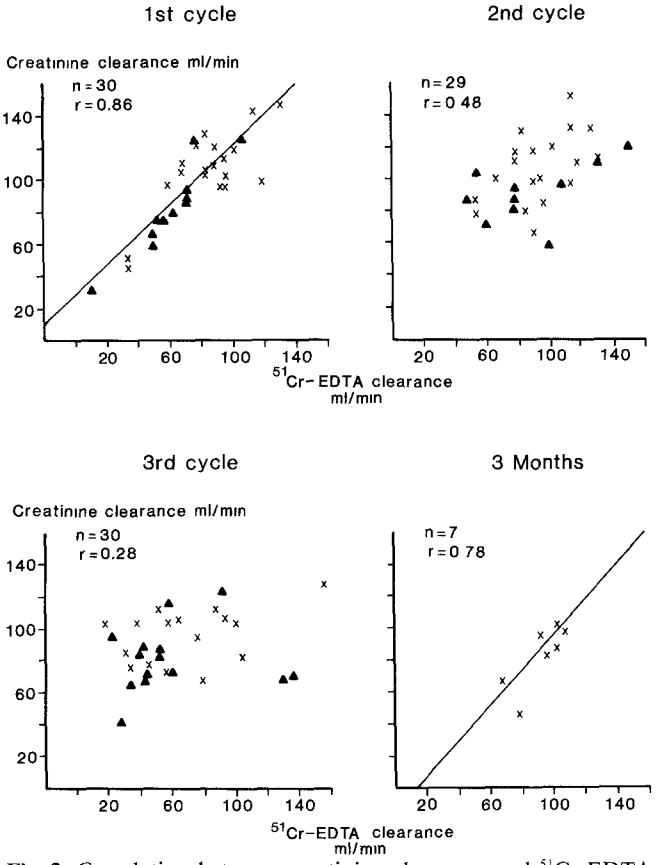

Fig. 3. Correlation between creatinine clearance and ^{51}Cr-EDTA clearance. x = values day 0 and ▲ values day 9

coefficient for the two variables went from high (r=0.86; slope of regression line = 0.91) during the first treatment cycle to low (r=0.28; slope of regression line = 0.16) during the third treatment cycle (Fig. 3). Three months after termination of treatment, a significant correlation be-

Table 2. Urinary excretion rates of beta-2-microglobulin (b-2-m), albumin, and IgG, and the fractional clearance ratios of b-2-m/albumin and IgG/albumin

		Urinary excretion of			Clearance ratios of	
		b-2-m mg/24 h	albumin mg/24 h	IgG mg/24 h	b-2-m/albumin	IgG/albumin
1st cycle	day 0	0.04± 0.02	2± 1	0.2± 0.2	421± 122	0.01±0.01
	day 6	18.94± 8.83*	80± 37	2.1± 1.1	31651±30073*	0.05±0.03
	day 9	0.16± 0.08	581±176*	66.7±25.1*	7± 3*	0.18±0.06*
	day 16	1.82± 1.03*	164± 79	8.2± 7.6	224± 121	0.04±0.02
2nd cycle	day 0	0.07± 0.03	20± 12	0.5± 0.4	270± 139	0.01±0.01
	day 6	38.54±19.38*	119± 56	11.2±10.3	9607± 5082*	0.06±0.05
	day 9	0.14± 0.03	552±241*	50.8±31.0	9± 5*	0.16±0.05*
	day 16	1.26± 1.16	183± 64	4.5± 2.4	201± 143	0.04±0.03
3rd cycle	day 0	0.17± 0.08	54± 15**	1.5± 1.5	100± 78	0.06±0.06
	day 6	10.64± 2.70*	252± 62	0.8± 0.8	11932± 7029*	0.01±0.01
	day 9	0.12± 0.08	310±142	56.3±20.6*	8± 4*	0.36±0.08*
	day 16	9.32± 6.83	208± 81	41.8±31.4	370± 260	0.28±0.16

* $P < 0.05$ compared to day 0 within the same cycle
** $P < 0.05$ compared to day 0 in the 1st cycle

tween creatinine clearance and ^{51}Cr-EDTA clearance was found again ($r=0.78$; slope 1.05).

Low-dose therapy. The comparison between high- and low-dose regimens showed that after a cumulative dose of 200 mg/m^2 cisplatin, the ^{51}Cr-EDTA clearance decreased significantly more ($P < 0.05$) in the high-dose group (15.1 ± 3.6 ml/min) than in the low-dose group (6.8 ± 2.2 ml/min). After 400 mg/m^2, the decrease was 30.5 ± 2.9 ml/min and 12.8 ± 2.6 ml/min, respectively ($P < 0.0005$).

S-urea increased significantly during each treatment cycle but reached normal values before the next cycle. However, 3 months after the last course it had reached a significantly elevated level, i.e., $43 \pm 11\%$ above pretreatment values.

Table 2 presents the urinary excretion rates of b-2-m, albumin, and IgG and the clearance ratios of b-2-m/albumin and IgG/albumin. A significant increase was observed in the b-2-m urinary excretion rate on day 6, and at the same time a significant increase in the b-2-m/albumin clearance ratio was observed. This clearance ratio decreased significantly on day 9 in all three cycles. The urinary excretion rate of IgG increased on day 9, with a significant increase in the first and third cycles. At the same time, a significant increase in the IgG/albumin clearance ratio was obtained in all three cycles.

Discussion

The main finding of the present study was that the GFR suffers significantly during cisplatin treatment, and more so with large than with small single doses at the same cumulative doses. No nephrotoxicity for either VP-16 or bleomycin has previously been described [6], suggesting that the decrease in GFR in the present study can be attributed to cisplatin. Second, neither s-creatinine nor creatinine clearance reflects the deterioration of GFR as measured with ^{51}Cr-EDTA. Third, the proteinuria caused by cisplatin treatment is of a different character during and between treatment cycles.

Several authors have discussed whether the decrease in GFR depends on the amount of cisplatin delivered as a single dose or whether the decrease in kidney function depends on the cumulative dose delivered. The present study shows that after a cumulative dose of 400 mg/m^2, patients treated with low-dose cisplatin had a significant decrease in GFR that was significantly less than the decrease in patients treated with high single doses. This observation suggests that when cisplatin is given for 5 days the decrease in GFR is dependent on the size of the single dose. The decrease in ^{51}Cr-EDTA clearance occurred in spite of the use of 3% saline as the vehicle for cisplatin in the high-dose group. Thus, hypertonic saline as a vehicle for cisplatin does not seem to abolish the decrease in GFR, an observation in agreement with that of Legha et al. [17].

The present study clearly demonstrates that when the GFR undergoes dynamic changes, s-creatinine cannot keep pace and does not reflect the real situation in the transition phase. Figures 1 and 2 demonstrate this. The early rise in s-creatinine can be explained as being due to muscle wasting, and the subsequent decrease during the second and third treatment cycles might reflect a low production rate due to the loss of muscle mass. Actually, during three treatment cycles these young patients suffered an average weight loss of $10-35 \pm 1.04$ kg. However, 3 months after termination of treatment, body weight was normalized compared to the pretreatment value and a significant increase in s-creatinine was observed. Furthermore, s-creatinine values will tend to drag behind the actual changes in renal function, since it will take some time to fill the large distribution volume for creatinine in the body above normal concentration values. These are well-known facts, previously described in postoperative patients [14]. Also, it has previously been shown that s-creatinine is a very insensitive measure of changes in GFR when the latter occurs in a moderately reduced range [26], relevant for the present group of patients.

The fact that creatinine clearance stayed constant during the whole period may seem surprising, but many factors are known to affect this parameter, which is not a simple reflection of GFR. It has previously been shown that an increase in the absolute tubular secretion of creatinine in patients with a moderate reduction of GFR maintains creatinine clearance in a normal or near-normal range and serves to delay the predicted hyperbolic rise above normal values of the s-creatinine level as the GFR falls to approximately half normal [26]. Also, during rapid changes in renal function and during conditions with progressive muscle waste, a poor correlation between changes in GFR and creatinine clearance is observed. In the present study, an agreement between ^{51}Cr-EDTA clearance and creatinine clearance was found in the pretreatment phase with normal values, and in the late post-treatment phase with reduced values (Fig. 3). Between these phases, however, creatinine clearance is affected by changes in the production rate. The poor correlations between ^{51}Cr-EDTA clearance, s-creatinine, and creatinine clearance have also been described in children treated with cisplatin [30].

Proteinuria in combination with cisplatin treatment is not mentioned very frequently [2, 5, 15, 25, 27]. Flemming et al. [10] found a small increase in urinary albumin and IgG excretion 1–5 days after low-dose cisplatin treatment, but after different cumulative doses. In the present study, all patients developed some degree of proteinuria. Peterson et al. [22] observed a high b-2-m/albumin clearance ratio in tubular proteinuria, an intermediate in normal subjects, and a low ratio in glomerular proteinuria. Therefore, the data given in Table 2 suggest that the proteinuria occurring during the cisplatin infusion (day 6) is mainly of tubular origin, whereas the proteinuria observed between the infusions is mainly of glomerular origin.

Glomerular or renal vascular lesions have not been reported in combination with cisplatin treatment [11, 23]. The increase in the IgG/albumin clearance ratio might be related to biophysical influences such as hemodynamic and charge-selective characteristics of the glomerular capillaries, causing changes in the permeability of the glomerulus to macromolecules [24]. These alterations may not be detected by conventional electron microscopic methods that are used to examine diseased glomeruli.

In conclusion, a significant and permanent decrease in GFR as measured by ^{51}Cr-EDTA clearance is observed after treatment with high-dose cisplatin. Hypertonic saline does not abolish this decrease, which is dependent on the size of the single dose. S-creatinine and creatinine clearance are unsuitable as measures of kidney function in this group of patients.

The proteinuria observed during cisplatin infusion is predominantly of tubular origin, whereas the proteinuria occurring between the treatment periods is mainly of glomerular origin.

Acknowledgement. Thanks are due Mrs. Inge-Lise Rossing, Department of Clinical Physiology, The Finsen Institute, for excellent technical assistance.

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Received October 10, 1987/Accepted December 8, 1987