Eur J Cancer Clin Oncol, Vol. 21, No. 4, pp. 447-451, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

Nephrotoxicity of 1,1-Diaminomethylcyclohexane Sulphato Platinum II (Spiroplatin; TNO-6)*

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Abstract—*1,1-Diaminomethylcyclohexane sulphato platinum II (spiroplatin; TNO-6) is a new platinum-containing analog used as an antitumor agent. Renal function studies were performed in eight patients treated with this drug (30 mg/m²). During and after the first spiroplatin infusion there was a decrease in effective renal plasma flow (ERPF: $P < 0.05$) and an increase in filtration fraction ($P < 0.01$) without changes in glomerular filtration rate (GFR), suggesting changes in renal hemodynamics. During the same period there was an increase in relative N-acetyl-β-D-glucosaminidase excretion ($P < 0.01$), pointing to tubular cell damage. Also, an increase in relative β_2-microglobulin excretion was established during and after spiroplatin infusion. On day 21 a decrease in GFR was found (median 7.4%), together with a decrease in ERPF (median 11.8%).*

INTRODUCTION

SINCE its development in 1965 by Rosenberg *et al.* [1], *cis*-diamminedichloroplatinum II (cisplatin; CDDP) has become a widely used antitumor agent against a variety of cancer types [2, 3]. One of its major side-effects is the deterioration of the renal function [4, 5]. Therefore, many platinum-containing analogs with the same or increased anti-tumor effect in animals but with less side-effects as compared to the original substance CDDP, and especially with less nephrotoxicity, have been or will be evaluated in man.

1,1-Diaminomethylcyclohexane sulphato platinum II (spiroplatin, TNO-6) is one of these new platinum-containing antitumor agents. In order to evaluate its nephrotoxicity, we performed renal function studies in eight patients treated with spiroplatin in a phase II trial.

MATERIALS AND METHODS

Eight patients who were entered in a multicenter phase II trial were studied. Five patients were male, mean age 52 yr (range 46–56 yr); three patients were female, mean age 46 yr (range 28–69 yr). The patients had different tumor types. All patients had a serum creatinine level < 120 μmol/l and/or a creatinine clearance ≥ 60 ml/min. None of the patients had been pretreated with cisplatin or other platinum-containing analogs. All patients received spiroplatin at a dose of 30 mg/m². Spiroplatin was supplied by Bristol-Myers International, Brussels, in vials containing 10 mg of the drug. The total dose was given over a period of 4 hr intravenously in 500 ml 5% glucose from noon until 4 p.m. Treatment courses were repeated after 3 weeks.

Renal function studies consisted of a simultaneous measurement of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), and a calculation of the filtration fraction (FF). These variables were studied before, during and for 4 hr after the first spiroplatin infusion. From 9 a.m. until 7 p.m. supine GFR and ERPF were measured hourly with [^{125}I] sodiumiothalamate and [^{131}I]hippuran, respectively, as described previously [6]. Errors in GFR introduced by incomplete collection of urine were corrected [6]. FF was calculated as the quotient of GFR and ERPF. The variation coefficient of the GFR and ERPF determinations amount to 2.2 and 5% respectively.

Urine was collected hourly for the determination of *N*-acetyl-β-D-glucosaminidase (NAG) and

Accepted 1 October 1984.
*This study was supported by grant C82-372 of the Dutch Kidney Foundation (Nier Stichting Nederland).

β_2-microglobulin from 9 a.m. until 7 p.m. Spiroplatin was infused from noon until 4 p.m. The pretreatment values for NAG and β_2-microglobulin excretion, as for GFR and ERPF, were calculated from the values of 9 a.m. to noon (prior to spiroplatin infusion).

At day 21, before the second infusion of the drug, GFR and ERPF determinations were repeated.

The spectrophotometric assay for urinary NAG activity was performed according to a slightly modified method of Horak *et al.* [7]. This assay includes three steps: gel-filtration chromatography on Sephadex G-25-M to separate NAG from urine inhibitors, followed by enzymatic hydrolysis of *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide in sodium citrate buffer for 15 min at pH 4.4 and spectrophotometry of *p*-nitrophenylate ion at 405 nm.

β_2-Microglobulin concentration in the urine was determined by a radioimmunosorbent technique according to Evrin *et al.* [8]. All urine samples had a pH >5.8 at voiding.

Creatinine concentration in the urine was determined with standard automatic technique. Next the ratio of NAG and creatinine (U/g) and of β_2-microglobulin and creatinine (μg/g) were calculated.

Statistics

Statistical analysis was performed with Wilcoxon's test for paired observations (two-sided).

RESULTS

The pretreatment values of GFR, ERPF and FF are listed in Table 1(a). Values are not corrected for body surface area. On day 1 the nadir ERPF was determined together with the corresponding values of GFR and FF. During and 4 hr after the spiroplatin infusion a decrease in ERPF (median decrease 16.4%, $P<0.05$) was found together with a slight increase in GFR (median increase 3.3%, N.S.). This resulted in an increase in filtration fraction in all patients [median increase 18.2% $P<0.01$; Table 1(b)].

On day 21 there still were decreases in median ERPF (11.8%) and in median GFR (7.4%), which, however, were not significant. In four patients a decrease in GFR of more than 5% was present and in two patients GFR fell more than 15%. The absolute values on day 21 are given in Table 1(c). Median FF on day 21 returned to the pretreatment value (only a slight increase of 4.5%).

Before, during and 4 hr after the spiroplatin infusion, urinary excretion of NAG, β_2-microglobulin and creatinine were determined. For NAG there was a marked increase in relative excretion during and after spiroplatin infusion in six patients ($P<0.01$; Fig. 1). During the same period also an increase in relative β_2-microglobulin excretion was found in these individuals. The mean values for relative NAG, β_2-microglobulin and absolute creatinine excretion before, during and after spiroplatin infusion are given in Table 2.

DISCUSSION

The clinical success of cisplatin treatment has led to the development of several hundreds of analogs. The rationale for developing new platinum compounds is to search for a compound that in comparison to cisplatin has a broader spectrum of antitumor activity, a lack of cross-resistance to cisplatin and a decreased toxicity (especially nephrotoxicity) [9–12].

One of these new analogs is diaminomethylcyclohexane sulphato platinum II (spiroplatin). Lee *et al.* [13] showed that spiroplatin had

Table 1. *Changes in GFR, ERPF and FF before, during and after spiroplatin infusion*

Patient No.	GFR (ml/min) a	b	c	ERPF (ml/min) a	b	c	FF a	b	c
1	62	72	77	291	290	329	0.21	0.25	0.23
2	57	50	70	274	219	235	0.21	0.23	0.30
3	132	173	138	554	568	588	0.24	0.31	0.23
4	126	129	116	568	470	493	0.22	0.27	0.23
5	199	204	165	627	625	547	0.32	0.33	0.30
6	111	103	109	476	436	450	0.23	0.24	0.24
7	119	122	110	569	532	498	0.22	0.23	0.22
8	151	133	124	649	468	574	0.23	0.26	0.22
Median	122	126	113	561	469	495	0.22	0.26	0.23

a. Values before spiroplatin infusion.
b. Nadir values of ERPF during and after spiroplatin infusion, together with the corresponding values of GFR and FF.
c. Values for GFR, ERPF and FF on day 21.

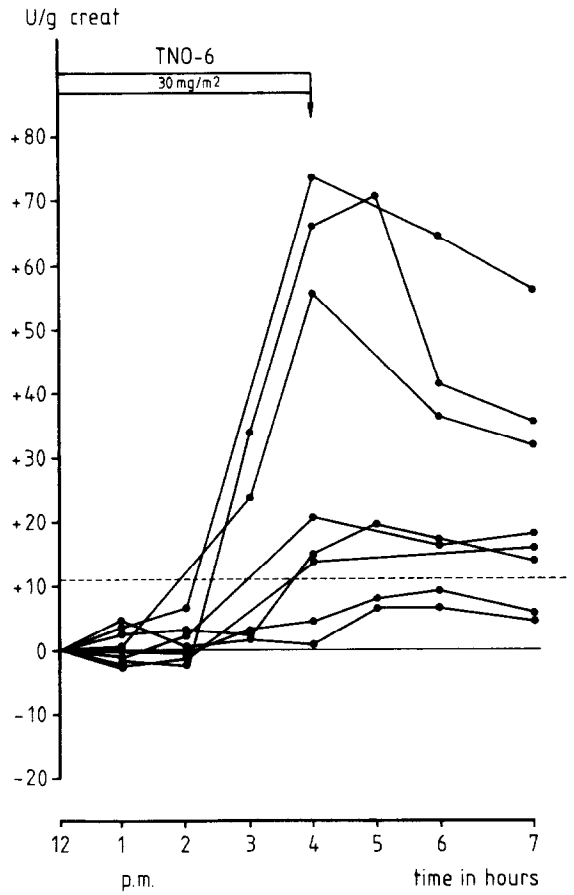

Fig. 1. Absolute change in relative N-acetyl-β-D-glucosaminidase excretion during and after the first spiroplatin infusion. The dotted line marks an increase of 11 U/g, the maximal increase found during cisplatin infusion.

comparable or even superior antitumor activity against L1210 leukemia in mice compared to cisplatin. However, the agent was inferior to cisplatin in B16 melanoma, Lewis lung carcinoma and Madson 109 lung carcinoma. In studies of De Jong *et al.* in rats [14] a similar antitumor activity of spiroplatin and cisplatin was established. Less nephrotoxicity was observed with spiroplatin (BUN, serum creatinine and renal histology) in both studies [13, 14] than with cisplatin.

In phase I studies Pinedo *et al.* [15] described as side-effects of spiroplatin nausea, vomiting, diarrhea, dry mouth and myelosuppression. After a bolus injection of spiroplatin proteinuria developed, decreasing with increasing duration of infusion; serum creatinine, however, remained stable in doses up to 30 mg/m² [16, 17]. Also, in a phase I study Groth *et al.* found no significant change in renal function in patients treated with spiroplatin during five consecutive days [18].

In a phase II trial Vermorken *et al.* [19] described an increase in serum creatinine in 15% of the patients. Franks *et al.* [20] mentioned six patients with acute renal failure and 12 patients with significant changes in renal function in phase II trials consisting of 239 patients (7.5% nephrotoxicity).

In the present study we first determined the acute effects of spiroplatin (30 mg/m²) on renal function and found a decrease in ERPF together with a significant increase in filtration fraction without changes in GFR. These effects can be explained by hemodynamic alterations in the kidney. Similar alterations were also found in cisplatin-treated patients, although in these patients only 20 mg/m² of the drug was administered on day 1 [21-23].

In the urine collected before, during and until 4 hr after spiroplatin infusion, *N*-acetyl-β-glucosaminidase (NAG) and β_2-microglobulin were determined. NAG is a hydrolytic enzyme mainly located in the lysosomal fraction of the renal tubular cell. The molecular weight of NAG is 130,000-140,000, so the enzyme does not pass through the glomeruli [24]. We found a rise in relative NAG excretion during spiroplatin infusion, in most patients exceeding the maximal increase during cisplatin infusion 20 mg/m² daily × 5 (unpublished results).

β_2-Microglobulin is a small, circulating protein with a molecular weight of 11,800, which is filtered by the glomerulus. After filtration 99.9% is normally re-absorbed. Malfunction of the proximal tubular cell leads to an impaired reabsorption mechanism resulting in increased β_2-microglobulin excretion [24]. We also found a rise in relative β_2-microglobulin excretion during and after spiroplatin, again in contrast with our experience with cisplatin [21].

On day 21 we found both a decrease in ERPF

Table 2. Changes in mean values of relative N-acetyl-β-D-glucosaminidase (NAG) and relative β_2-microglobuline (β_2-M) and absolute creatinine excretions before, during and after spiroplatin infusion

	NAG (U/g creatinine)	β_2-M (μg/g creatinine)	Creatinine (mmol/l)
Pretreatment	12.0	242.9	3.0
During spiroplatin	24.1	608.3	3.0
After spiroplatin	37.7	754.3	10.8

and GFR (median decrease of 11.8 and 7.4% respectively.

In conclusion, during spiroplatin infusion we found changes in renal hemodynamics which consisted of a decrease in ERPF with an increase in filtration fraction. Qualitatively, these changes are similar with those found with cisplatin. Further, an increase in relative excretion of NAG and β_2-microglobulin was established, suggesting acute renal tubular damage. This form of toxicity was more marked with spiroplatin than with cisplatin, which might be due to the relative difference in the doses. The initial changes in renal hemodynamics and tubular function may result in glomerular filtration later on [25]. Spiroplatin therefore seems to be potentially nephrotoxic on various levels in the kidney.

Acknowledgements—We wish to express our appreciation to Willy Bruins-van der Weij and Aly Drent-Bremer for their assistance.

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