ONCOLOGY

Modern Methods for Diagnosis and Monitoring of Nephrotoxicity during Antitumor Therapy with Platinum Derivatives

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Chemotherapy is an important method of antitumor therapy. Nephrotoxicity characteristic of many cytostatics, including platinum compounds, is a serious complication limiting the drug dose [4,5,7-9]. Platinum derivatives, in particular, cisplatin, are drugs of choice in the treatment of the majority of solid tumors. However, cisplatin induces a dose-dependent toxic damage to the kidneys [5,7,8,10].

Key Words: *chemotherapy; platinum preparations; nephrotoxicity; urinary proteins and enzymes*

Second-generation platinum derivatives are alternative preparations for tumor therapy due to their low nephrotoxicity. One of them is Russian-made drug cycloplatam [2]. Experiments demonstrated that, unlike cisplatin disturbing glomerular filtration and tubular secretion, cycloplatam little affect renal function.

Radionuclide tests (dynamic renoscintigraphy) based on plasma clearance from nephrotropic radiopharmaceutical (RP) are widely used at the Cancer Research Center for evaluating renal function and urodynamics. Complex renoscintigraphy (CR) [1], developed for chemotherapy monitoring, helps to evaluate one of the main processes in the kidney, concentration, which depends on both the glomerular and tubular function.

Routine laboratory tests (urine analysis and measurement of serum concentrations of urea and creatinine) are insufficiently informative for the diagnosis of toxic injuries to the kidneys. At present, measurements of urinary proteins and enzymes as highly sensitive markers of glomerular (albumin) and tubular (α_1-microglobulin, N-acetyl-β-D-hexosaminidase, γ-glutamyl transferase) dysfunction of the kidneys are widely used for evaluating renal injuries caused by antitumor cytostatics [3,6,9,11].

We evaluated the efficiency of CR and measurements of serum and urinary biochemical parameters during chemotherapy with first- and second-generation platinum derivatives for diagnosis and monitoring nephrotoxicity.

MATERIALS AND METHODS

Nephrotoxicity of chemotherapy protocols including cisplatin and cycloplatam was studied using the renal radioisotope test (CR) and biochemical analysis of the blood and urine in 25 patients (aged 39-72 years) with ovarian cancer (stages III-IV). None of the patients received chemotherapy before.

The patients were randomly divided into 2 groups: group 1 patients (n=9) received a single dose of cisplatin (100 mg/m^2) with cyclophosphamide (600 mg/m^2) and group 2 (n=16) cycloplatam (100 mg/m^2) on days

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1-4 and cyclophosphamide (600 mg/m^2) on day 5. Chemotherapy was stopped after aggravation of nephro-, neuro-, and hematotoxicity. Cisplatin was injected intravenously by drop infusion in parallel with water load (2.0-2.4 liters of Ringer's solution and/or 0.9% NaCl), cycloplatam intravenously by drop infusion in 400 ml 5% glucose. Chemotherapy courses were repeated every 4 weeks. The patients were observed for 2 weeks to 7 months and received a total of 1-7 courses.

Renal function and urodynamics were evaluated by CR (by the DIAS technology developed at the Cancer Research Center) with Russian RP technefor (^{99}Tc). Tests with low doses of RP (effective equivalent dose 0.3 mSv) were performed before the 1st, 3rd or 4th course, and after chemotherapy. The following parameters were evaluated: relative renal bloodflow Q_L-Q_R (normal value 45-55%); index of RP concentration in the parenchyma G (normally 30-50 arb. units) reflecting integral filtration-reabsorption clearance; rate of RP elimination from the cortex D (normal value 65-75%); and latency and rate of evacuation of labeled urine from the pelvis, T_B (normal value 5-8 min) and U (normal value 50-75%), respectively. In addition, radionuclide tests were used to reveal fluid and adhesions in the abdominal cavity and pelvis; preclinical signs of cystitis were also detected. CR was performed on a Siemens rota-chamber in two projections.

Biochemical analyses of the serum (creatinine and urea) and urine (protein and enzyme excretion) were performed before, during, and after chemotherapy. Creatinine clearance was evaluated before each course. Proteins and enzymes were measured in the second morning portion of the urine and the values were correlated to creatinine level in the analyzed sample. Proteinuria was assessed by the concentration of total protein, albumin, and α_1-microglobulin. Activities of enzymes with different subcellular location in the proximal renal tubular epithelium were measured: lysosomal; N-acetyl-β-D-hexosaminidase (NAG) and membrane bound γ-glutamyl transferase (γ-GT). Analyses of the urine and blood were performed by optimal spectrophotometric methods on a Hitachi-911 automated analyzer.

RESULTS

According to CR, the function of renal parenchyma in both groups was virtually the same: slight suppression (G=26-28) was observed in 1 of 9 patients treated with cisplatin and in 3 of 16 treated with cycloplatam. Urodynamic disorders of different degree (U<30%, D<50%) caused mainly by the tumor and ascites were observed in 40% patients in both groups. Radionuclide signs of ascites were detected in 77 and 21% patients treated with cisplatin and cycloplatam, respectively.

Chemotherapy was associated with deterioration of renal function (according to CR), due to immediate toxic effect of cytostatics, the degree of this deterioration depended on the obstruction. Deviations in the concentration capacity of the kidneys (G) were observed in 7 (78%) of 9 patients treated with cisplatin and in 7 (58%) of 12 treated with cycloplatam. The decrease in G value was statistically significant (about 24%, p<0.01) in the cisplatin group and minor (6%, p>0.05) in the cycloplatam group. After therapy G returned to the initial level in 4 (45%) and 5 (42%) patients, respectively. One case with asymmetric deterioration of G parameter during treatment was observed in each group, with initially symmetrical function of the parenchyma. The deterioration was more expressed in the cisplatin group, where the G value did not return to the initial symmetry after treatment. It is noteworthy that the maximum deterioration of parenchymal function (100 mg/m^2) was observed on the side of stable significant urodynamic disorders (U<5%, T_B>18 min).

TABLE 1. Serum Concentrations of Urea and Creatinine and Creatinine Clearance in Patients with Ovarian Cancer Treated with Cisplatin and Cycloplatam ($M\pm m$)

Parameter	Normal value	Cisplatin		Cycloplatam	
		initial	after treatment	initial	after treatment
Urea, mmol/liter	1.7-8.0	4.6±0.4	7.5±0.4*	4.6±0.3	5.8±0.3**
		(1.9-5.8)	(2.6-16.7)	(2.5-6.3)	(2.8-12.0)
Creatinine, mmol/liter	45-115	84.1±4.7	100.1±3.6**	81.3±3.7	85.6±1.9
		(60-103)	(63-156)	(55-104)	(65-104)
Creatinine clearance, ml/min	>100	73.6±4.3	58.4±2.7**	77.3±4.5	78.0±2.5
		(57-97)	(30-106)	(50-110)	(50-104)

Note. *p<0.001, **p<0.01 compared to the initial values.

TABLE 2. Urinary Protein and Enzyme Concentrations (U/mmol Creatinine) in Patients with Ovarian Cancer Treated with Cisplatin (*M±m*)

Parameter	Normal values	Initial values	Treatment, day		
			1	2-3	4
α_1-Microglobulin	<1.58	1.5±0.4 (0.44-2.8)	6.4±0.8 (0.9-17.3)	6.2±1.2 (1.5-14.6)	9.5±2.4 (1.1-60.6)
Albumin	<2.26	1.0±0.4 (0.44-2.8)	10.1±3.6 (0.4-89.9)	5.4±1.3 (0.6-14.9)	13.6±2.9 (0.7-69.9)
Total protein	<12.0	11.5±1.2 (0.9-17.6)	24.5±3.7 (8.1-82.3)	20.3±2.2 (11.0-28.0)	25.6±3.6 (2.4-71.3)
γ-GT	<0.6	5.I±0.27 (2.0-6.9)	11.3±1.1 (4.9-24.2)	5.8±0.47 (2.4-8.7)	6.1±0.5 (3.7-16.5)
NAG	<0.5	1.2±0.10 (0.5-2.8)	2.6±0.3 (1.1-6.2)	2.5±0.5 (1.3-7.5)	3.2±0.7 (0.9-18.0)

The function of each kidney separately as a functional unit was evaluated by CR. Maximum (12%) decrease of *G* in comparison with the initial level was observed during cycloplatam treatment in kidneys with normal or negligibly decreased urodynamics (*U*>30%), while in the kidneys with initially moderate or significantly impaired urodynamics (*U* below 30%) this parameter decreased by 22% ($p>0.05$). In cisplatin group the maximum decrease of *G* was 16% in the kidneys with initially normal urodynamics and 39% ($p<0.01$) in the kidneys with moderate and considerable urodynamic disorders. This suggests that initially increased pressure in the pelviocaliceal system of the kidney caused by mechanical compression of the ureters by the tumor, ascites, or adhesions provided conditions for higher nephrotoxic response of the parenchyma, but the decrease in the function of the parenchyma was more pronounced during cisplatin treatment.

The levels of urea and creatinine, proteinuria and hematuria were initially normal in all patients. Decreased creatinine clearance (57 ml/min) before treatment was observed in 1 of 9 patients in the cisplatin group and in 3 in the cycloplatam group (50-58 ml/min), which could be explained by inoperable tumor, ascites, and pleurisy in these patients.

Serum concentration of nitrogen-containing compounds before treatment was normal (Table 1). During cisplatin treatment azotaemia increased in 77.8% cases; some patients had essentially increased concentrations of urea and creatinine corresponding to first-degree nephrotoxicity according to WHO criteria (Table 1). The mean levels of urea and creatinine after treatment increased 1.6- and 1.2-fold, respectively ($p<0.001$ and $p<0.01$). Moreover, the clearance of creatinine notably ($p<0.01$) decreased in the majority (88.8%) of patients in this group during chemotherapy, which indicated disturbed glomerular filtration. In the cycloplatam group, the increase of azotemia was less pronounced. Increased serum concentration of urea (to 12 mmol/liter) was detected in 3 (18.7%) patients and corresponded to first-degree nephrotoxicity. On the whole no significant decrease in creatinine clearance was observed in this group (Table 1).

Protein content and enzyme activities were normal before treatment (Table 2). The only exclusion was lysosomal hydrolase NAG, whose mean activity

TABLE 3. Urinary Protein and Enzyme Concentrations (U/mmol Creatinine) in Patients with Ovarian Cancer Treated with Cycloplatam (*M±m*)

Parameter	Initial values	Treatment, day		
		1	2-3	4
α_1-Microglobulin	1.3±0.4 (0.09-4.50)	6.00±1.82 (0.4-48.0)	20.9±2.7 (2.0-54.0)	13.4±3.2 (1.5-46.6)
Albumin	1.3±0.3 (0.3-2.2)	1.90±0.58 (0.2-17.2)	16.8±3.1 (0.5-84.8)	9.8±2.3 (0.5-49.6)
Total protein	11.7±1.6 (4.6-17.7)	15.0±1.9 (4.0-41.9)	61.4±8.9 (10.0-163.7)	38.7±6.2 (5.3-108.7)
γ-GT	5.7±0.3 (2.0-8.9)	6.2±0.4 (1.6-18.9)	8.7±0.6 (4.1-17.0)	9.0±0.9 (4.3-20.5)
NAG	1.3±0.2 (0.4-5.2)	2.4±0.6 (0.5-8.0)	4.4±0.6 (0.8-17.0)	3.0±0.5 (0.96-10.0)

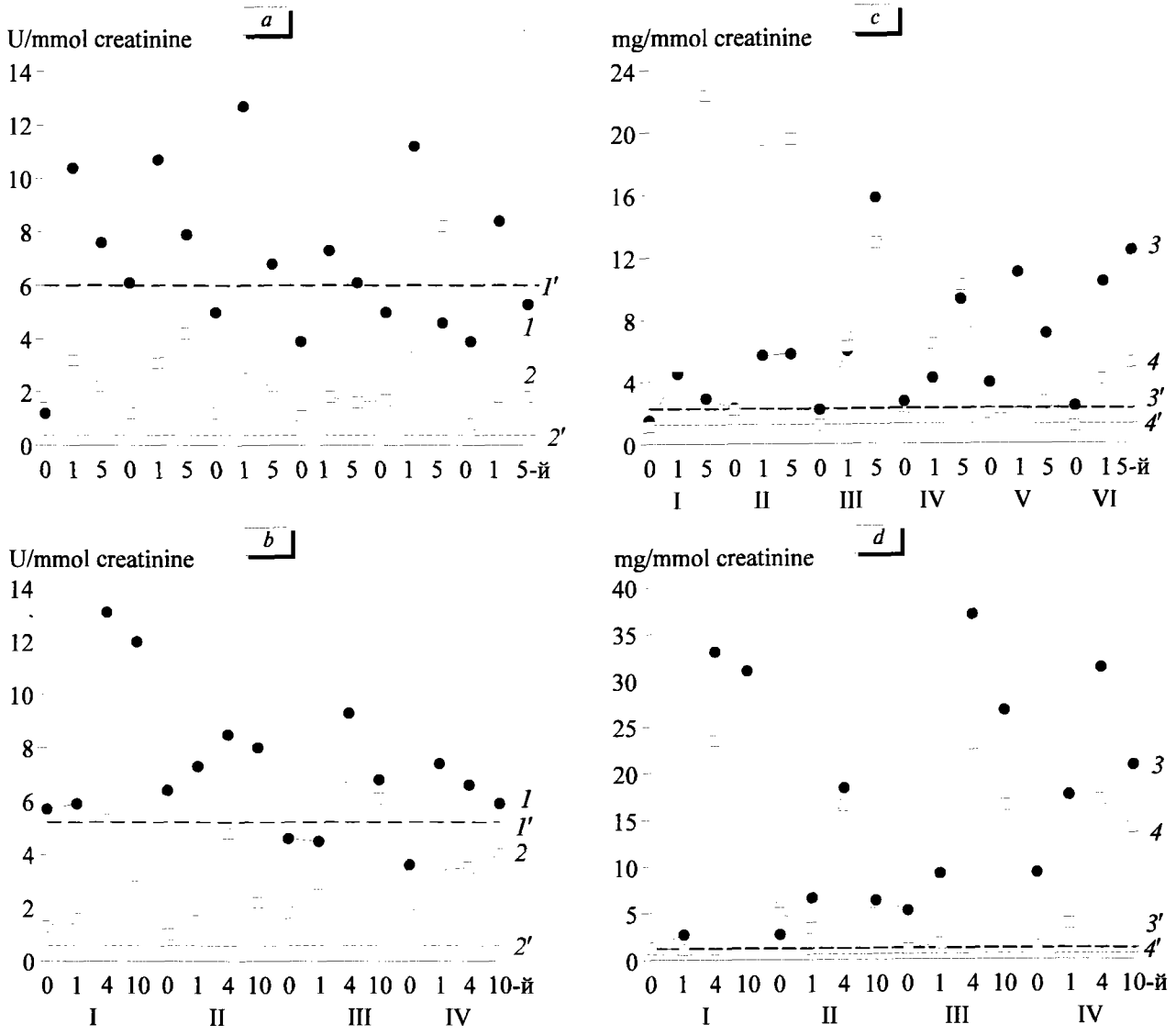

Fig. 1. Activities of enzymes (*a*, *b*) and protein content (*c*, *d*) in urine of patients with ovarian cancer treated with cisplatin (*a*, *c*) and cycloplatam (*b*, *d*). Here and in Fig. 2: *1*) γ-glutamyl transferase; *2*) N-acetyl-β-D-hexosaminidase; *3*) α_1-microglobulin; *4*) albumin. Abscissa: Chemotherapy course No. (Roman numerals), day of the course (Arabic numerals). Here and in Figs. 2 and 3 normal values are shown by dotted lines with respective figures.

in the urine of patients treated with cisplatin significantly ($p<0.001$) surpassed the norm, which can be explained by the presence of patients with residual large tumors in the pelvic and ascites causing intoxication and urodynamic disorders in this group. The detected regularities indicate the necessity of evaluating initial enzymuria and repeated measurements of urinary enzymes during treatment for subsequent adequate interpretation of the results.

Combined chemotherapy including cisplatin caused more stable hyperprotein- and hyperenzymuria than cycloplatam therapy. Excretion of proteins and enzymes increased after the first injection of cisplatin (Fig. 1). NAG activity in the urine increased and peaked on day 4-5 of chemotherapy and did not return to normal by the start of the second course, while urinary excretion of γ-GT peaked on day 1 after drug injection, gradually decreased by days 4-5, and returned to normal by the next course. Increase in urinary enzyme excretion after drug infusion was significant ($p<0.001$) in comparison with the respective initial levels in all studied terms. The only exclusion was γ-GT, whose urinary excretion increased significantly only on day 1 postinjection, which can be explained by its cellular localization (γ-GT in cell membrane and of NAG in the nephrothelial lysosomes).

Protein concentration in the urine increased significantly on day 1 after infusion of cisplatin and reached the maximum by day 4-5 (Table 2). Increase of proteinuria was significant for all studied parameters, the maximum changes being characteristic of α_1-microglobulin and albumin. It is noteworthy that protein

concentrations always returned to normal by the next course of chemotherapy.

Though the mean levels of enzymuria, proteinuria, and serum concentration of nitrogen-containing compounds did not significantly increase from one course to another, and moreover, almost always normalized, except NAG, 2 patients with nephrotoxicity of the first degree developed a tendency to progressive increase in all parameters during chemotherapy, which was paralleled by the development of clinical manifestations of renal dysfunction (absence of urination, proteinuria, hematuria, arterial pressure rise).

Enzymuria and proteinuria increased in patient M. during the first courses of cisplatin therapy (Fig. 2), while serum concentration of creatinine increased only after the 5th course in the presence of clinical symptoms of renal failure, and therefore proteins and enzymes (particularly NAG) in the urine were early nephrotoxicity markers. The patient had stable urodynamic disorders initially and during chemotherapy, which led to irreversible moderate decrease in the parenchymatous function of both kidneys during therapy with high cisplatin doses (Fig. 3).

Cycloplatam treatment also led to high urinary enzyme and protein excretion, which were less stubborn than in cisplatin treatment. Serial studies showed that after the first infusion of cycloplatam the excretion of enzymes and proteins increased and peaked by day 4 (Table 3). Urinary levels of protein and enzymes gradually increased with accumulation of cycloplatam dose from day 1 to day 4, the increase in enzymuria and proteinuria on day 4 of the course being significant in comparison with their initial values. Later enzyme and protein excretion decreased and returned to the initial level by the start of the next course (Fig. 1).

A statistically significant increase in urinary concentrations of enzymes and proteins in comparison with the initial level was observed after the end of treatment (day 10). Presumably high enzyme- and proteinuria on days 4-10 of cycloplatam treatment were ex-

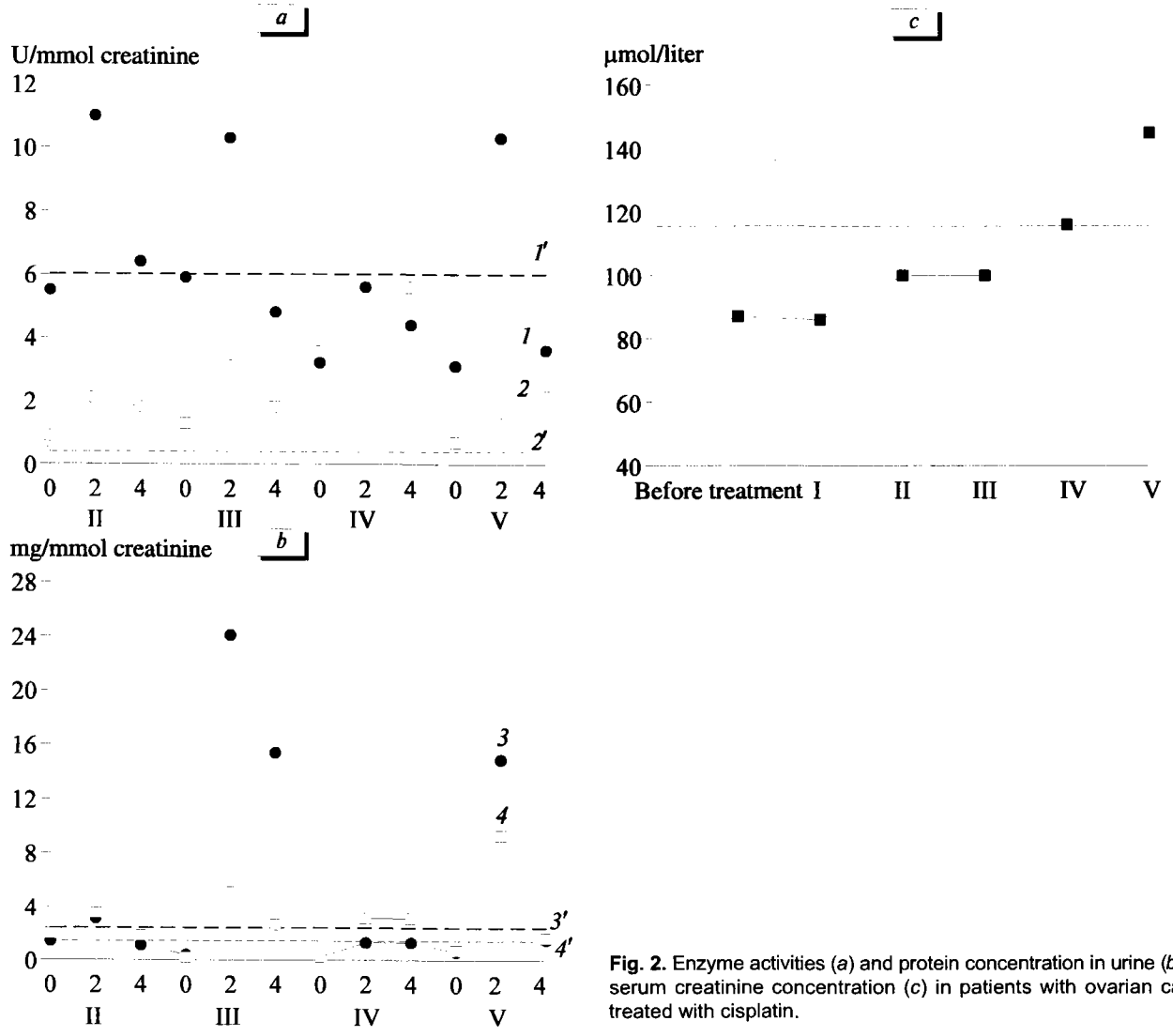

Fig. 2. Enzyme activities (*a*) and protein concentration in urine (*b*) and serum creatinine concentration (*c*) in patients with ovarian cancer treated with cisplatin.

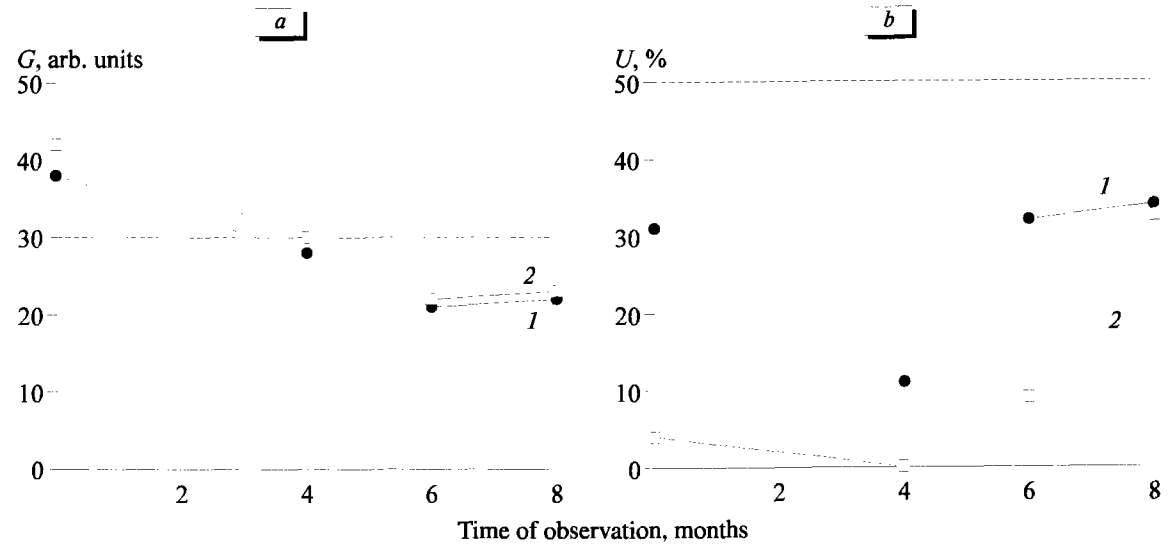

Fig. 3. Time course of radiopharmaceutical concentration in the parenchyma (*G*) and rate of urine excretion from the pelvis (*U*) in the left (*1*) and right (*2*) kidneys of the same patient. Cisplatin treatment, rehabilitation from month 6 to month 8.

plained by the drug metabolism and its accumulation by day 4 of treatment in the absence of forced diuresis.

Enzyme and protein excretion with the urine was characterized by high individual variability. The most pronounced changes were characteristic of NAG and α_1-microglobulin levels reflecting the status of proximal tubules and of albumin whose content reflected the function of the renal glomeruli. NAG activity in some patients treated with cycloplatam reached very high values (10-17 U/mmol creatinine) which were compatible with those during cisplatin treatment (7.5-18.0 U/mmol creatinine). The increase in urinary concentrations of α_1-microglobulin and albumin was high during both cisplatin (4.1-6.3 and 5.4-13.6 times, respectively) and cycloplatam (4.6-16.0 and 1.5-12.9 times, respectively) treatment.

Tumor location and dissemination in the abdominal cavity and pelvis, the presence of ascites, and concomitant diseases, including renal pathology, could also affect protein and enzyme excretion with the urine. The increase in protein and enzyme concentrations in the urine varied within a wide range in different patients, which could be explained by different sensitivity to cytostatics and reflects the severity of renal involvement. A considerable increase in NAG excretion was observed in all patients with toxic involvement of the kidneys during repeated cisplatin courses, the increase in the enzyme activity being stable without normalization. Thus, measurements of NAG as the most sensitive marker of renal involvement [6] are therefore an important test.

The detected shifts indicate the toxic effects of platinum preparations on the kidneys, which was confirmed by radionuclide monitoring. Cycloplatam affects mainly the tubular system of the kidneys, while cisplatin disturbs both the tubular and glomerular functions. Hence, screening of urodynamic disorders by CR is important for predicting the nephrotoxic effect of cisplatin and individual selection of water regimen for cycloplatam therapy in patients with ovarian cancer.

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