The Significance of Enzymuria in Assessing the Nephrotoxicity of Antitumor Chemotherapy in Children

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The significance of enzymuria in assessing the nephrotoxicity of cisplatin in a total dose of 100 mg/m^2 or iphosphamide in a total dose of 9 g/m^2 for children with solid malignant tumors (10 and 9 patients, respectively) was studied. Chemotherapy caused stable hyperenzymuria consisting in a significant increase in N-acetyl-β-D-hexosaminidase, γ-glutamyltransferase, and alanine aminopeptidase activities in the urine during chemotherapy in comparison with the initial values. The levels of enzyme excretion with the urine were higher in patients treated with iphosphamide than in those treated with cisplatin. The increase in serum creatinine and urea concentrations *vs.* the age-specific norm was observed in only 2 out of 9 children treated with iphosphamide. These results permit considering enzymuria as the most sensitive method for the diagnosis of nephrotoxicity.

Key Words: *chemotherapy; nephrotoxicity; urine enzymes*

The therapeutic effect of antitumor drugs is often associated with toxic effects on various organs and systems. Nephrotoxicity characteristic of the platinum preparations, iphosphamide, and other cytostatics is a complication limiting the drug dose. Biochemical parameters routinely used for the diagnosis of toxic involvement of the kidneys, primarily nitrogen-containing serum compounds urea and creatinine, are not very informative. Measurements of the urinary enzymes have been recently used for the diagnosis of renal involvement caused by toxic effects of antitumor, antibacterial, and other drugs [1,6,8, 10]. Biochemical analysis of urine for early detection of nephrotoxicity of antitumor therapy is particularly important in pediatric oncology, since the proposed method is noninvasive.

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The aim of this study was to assess the significance of biochemical parameters of blood serum and urine for timely diagnosis of nephrotoxicity in children with solid tumors receiving chemotherapy.

MATERIALS AND METHODS

The nephrotoxicity of chemotherapy protocols including iphosphamide and cisplatin has been assessed by biochemical analysis of the blood and urine in 19 children (10 boys and 9 girls) aged 5-16 years before, during, and after chemotherapy. The children were followedup from 2 weeks to 3 months, during this period they were administered 1-3 5-day courses at 3-4-week intervals. Nine children with nephroblastoma relapses (n=3), nephroblastoma (n=2), rhabdomyosarcoma (n=3), and disseminated Ewing's sarcoma (n=1) were treated by iphosphamide-based protocols including iphosphamide in a total dose of 9 g/m^2 per course, vepeside (500 mg/m^2), and carboplatin (450 mg/m^2). Ten children with osteogenic sarcoma (n=4)

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and Ewing's sarcoma (n=6) were treated using protocols including cisplatin in a dose of 100 mg/m^2 per course, adriamycin (50-60 mg/m^2), and cyclophosphamide (1000 mg/m^2). Control group consisted of 19 healthy children and 31 patients with benign diseases of the skin and subcutaneous fat (lateral cysts of the neck, pigmented nevi) matched for age and sex.

Enzymes with different subcellular localization in the epithelium of the renal proximal canaliculi were measured: lysosomal enzyme N-acetyl-β-D-hexosaminidase (NAG) and membrane-bound enzymes of the nephrothelial brush border: γ-glutamyltransferase (γ-GT) and alanine aminopeptidase (AAP). The enzymes were measured in the second morning portion of urine. Creatinine concentration was measured in the same portion. The results were expressed as the enzyme activity units/mmol creatinine. Urea and creatinine concentrations were measured in the blood serum. The studies were carried out using optimal spectrophotometric procedures in a Hitachi-911 analyzer.

RESULTS

The activities of enzymes in children with solid tumors before chemotherapy were virtually the same as in the control group, in which the threshold values were for γ-GT 9.5, for AAP 4.4, and for NAG 0.6 U/mmol creatinine. Comparison of the results of measurements in the urine and serum during and after chemotherapy with iphosphamide and platinum preparations (Table 1) showed that both protocols generally led to stable hyperenzymuria. Urinary excretion of the enzymes increased as soon as after the first injection of the drugs; enzymuria progressed and reached the peak on days 4-5 of chemotherapy. A significant increase in enzymuria was observed for all the enzymes measured during the treatment with both iphosphamide (p=0.005-0.04) and cisplatin (p=0.0003-0.01). Table 1 shows that the level of NAG excretion on day 5 of chemotherapy was 19.4 times increased in the children treated with iphosphamide. The increase in the activities of membrane-bound enzymes was lower: 3.7 times for AAP and 1.9 times for γ-GT. Similar regularities were observed during cisplatin therapy, but the increase in NAG excretion was not so drastic (5.6 times), whereas for AAP and γ-GT it was more pronounced (7.9 and 2.7 times, respectively) in comparison with the iphosphamide group. Enzymuria decreased during the intervals between the courses, normalizing by the beginning of the next course (Fig. 1).

The increase in enzyme activities in the urine was individual: predominant excretion of this or that enzyme was observed. The type of excretion depended on the localization of the primary tumor and concomitant diseases. The degree of increase in the concentrations of excreted enzymes varied within a wide range: from 1.2 to 23.8 times, which can be explained by different sensitivity to cytostatics and thus can reflect the severity of renal involvement. Measurements of lysosomal NAG hydrolase are especially interesting; many scientists believe that it is the most sensitive marker of liver involvement [2, 4,7]. We also observed an increase in NAG excretion in all patients with pronounced nephrotoxicity during repeated courses of polychemotherapy including iphosphamide. In some children, NAG activity reached the values so high that were never observed before treatment (9.5-43.2 U/mmol creatinine). Urinary excretion of NAG in such children increased more than 15 times; a stable increase in NAG activity was observed, and this value never dropped to the initial level observed in the majority of patients during the intervals between the courses. On the other hand, the excretion of membrane-bound AAP and γ-GT was moderately (by 3.2-7.4 times) increased in the ma-

TABLE 1. Urinary Enzymes (U/mmol Creatinine) in Children before and during Chemotherapy with Iphosphamide and Cisplatin ($X\pm\sigma$)

Enzymes	Chemotherapy, days					
	iphosphamide			cisplatin		
	0	5	9	0	5	9
NAG	0.5±0.15	9.7±4.5	1.9±1.3	0.43±0.14	2.4±1.0	1.5±0.5
	(0.3—0.7)	(4.8—17.4)	(0.4—4.9)	(0.3—0.8)	(1.1—5.6)	(0.8—2.5)
AAP	2.1±0.7	7.7±3.4	6.2±2.9	1.9±1.1	15.0±5.9	4.2±2.1
	(1.1—3.1)	(4.0—14.6)	(2.4—9.9)	(0.8—5.3)	(6.9—2.6)	(1.5—8.1)
γ-GT	5.5±1.8	10.8±3.6	7.2±2.1	5.3±1.1	14.3±5.4	6.8±1.4
	(3.1—6.9)	(6.6—18.9)	(4.2—12.9)	(2.8—8.3)	(9.2—7.9)	(4.9—10)

Note. The range of enzyme activity is given in parentheses.

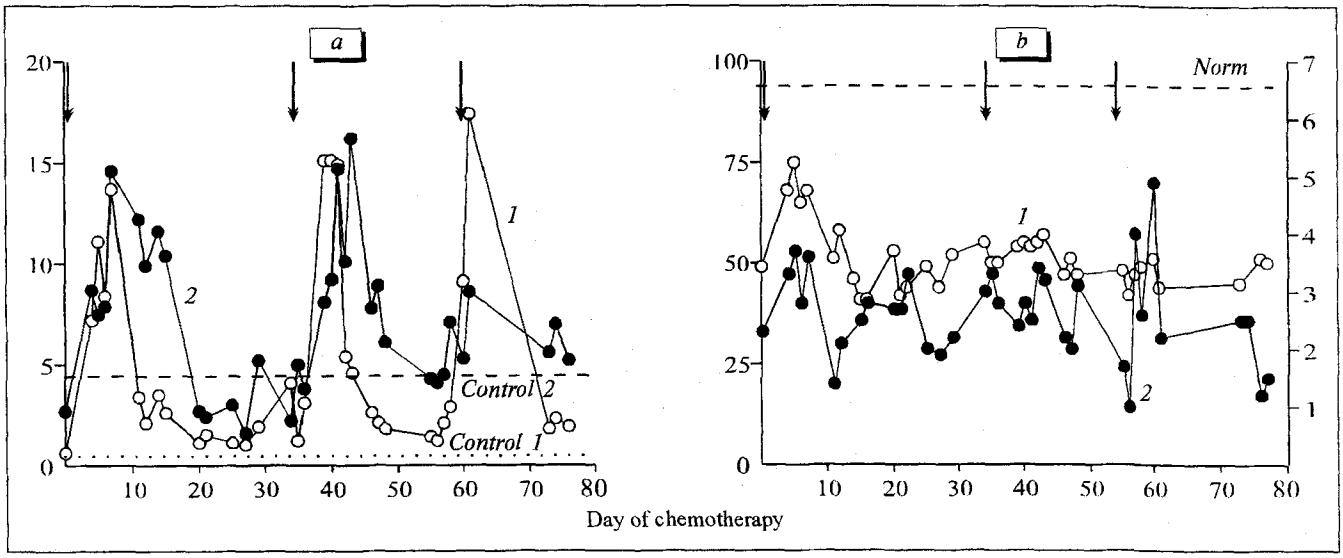

Fig. 1. Urinary enzymes (*a*), serum creatinine and urea (*b*) in girl P. with a relapse of nephroblastoma (No. 95/1791) treated with iphosphamide. Here and in Fig. 2: ordinate: *a*) activity of urinary enzymes, U/mmol creatinine; *b*) concentrations of creatinine, μmol/liter (left) and urea, μmol/liter (right). The arrow shows the beginning of chemotherapy. *a*: *1*) N-acetyl-β-D-hexosaminidase; *2*) alanine aminopeptidase. *b*: *1*) creatinine; 2) urea.

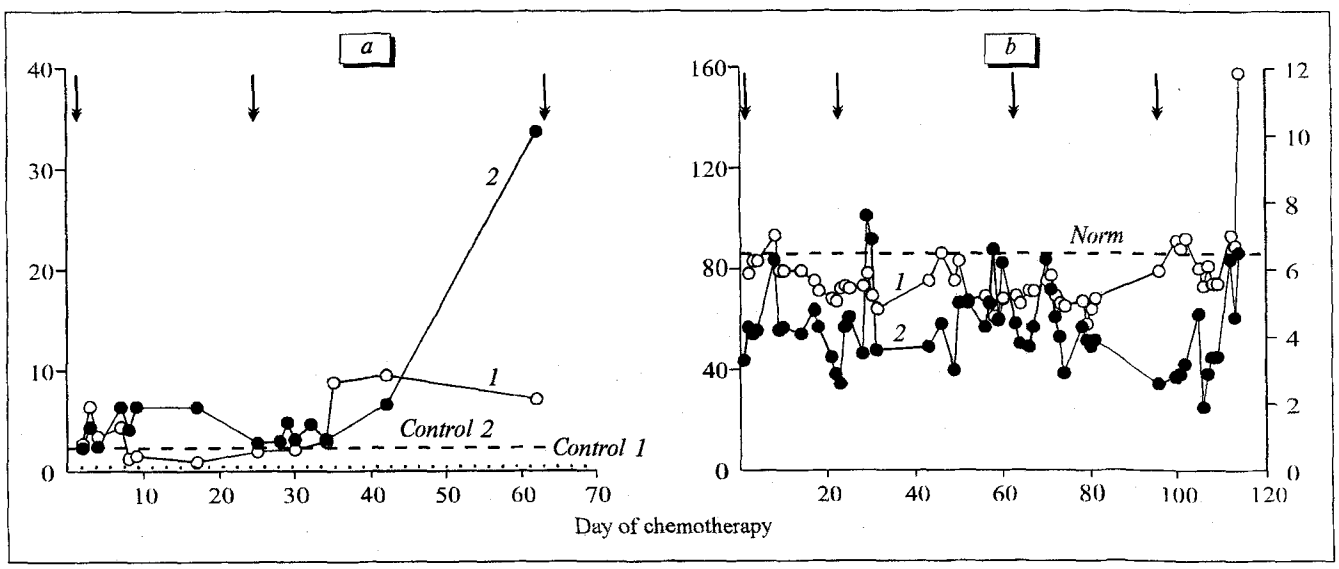

Fig. 2. Urinary enzymes (*a*), serum creatinine and urea (*b*) in boy A. with a relapse of nephroblastoma (No. 94/2061) treated with iphosphamide.

jority of cases; the increase in AAP excretion better reflected the toxic effect of therapy than of γ-GT. Figure 2 shows changes in enzymuria during chemotherapy of a nephroblastoma relapse in a boy aged 11 years. Urinary excretion of enzymes in comparison with the initial level (NAG — 0.6, AAP — 1.1 U/mmol creatinine) increased in response to the third injection of iphosphamide during the first course of chemotherapy: the activities of NAG and AAP corresponded to 6.4 and 4.3 U/mmol creatinine. Hyperenzymuria was stable during the entire follow-up and did not decrease by the beginning of the second course; after further injections of carboplatin and iphosphamide it became irreversible. Figure 2 shows the time course of serum creatinine and urea concentrations; their increases were negligible in comparison with the upper threshold age-specific values (88 μmol/liter for creatinine and 6.4 mmol/liter for urea). The level of creatinine increased once to 93 mmol/liter during the first course and then normalized; only during the fourth course its moderate (79-93 μmol/liter) increase was stable, with a sharp rise to 158 μmol/liter, which was paralleled by clinical signs of acute renal failure. The concentration of urea was virtually normal, with a negligible increase to 7.6 mmol/liter during the second course.

Measurements of nitrogenous compounds in the serum during antitumor treatment demonstrated their poor validity for the diagnosis of nephrotoxicity, because the concentrations of creatinine and urea were virtually normal in almost all the patients treated by nephrotoxic agents and increased only with development of renal failure. A moderate increase in serum nitrocompounds *vs.* the age-specific norm was observed in only 2 (22.2%) out of 9 children treated with iphosphamide. Pronounced hyperenzymuria preceded an increase in azotemia in all cases. The levels of creatinine and urea never increased beyond the normal for age level in any of the children treated with cisplatin over the entire follow-up. Figure 1 shows a typical time course of serum and urine biochemical parameters in a 6-year-old girl with a nephroblastoma relapse treated with iphosphamide. NAG and AAP in the urine and creatinine and urea in the serum were regularly measured. After three courses of chemotherapy, enzymuria markedly increased, reflecting a probable toxic effect of iphosphamide on the kidneys. Other signs of the condition were proteinuria, microhematuria, and decrease of renal function shown by renography. The creatinine and urea concentrations remained normal during the entire study. An increase in urinary enzymatic activities in the presence of normal values of nitrogenous compounds in the serum permits us to regard enzymuria as the earliest sign of nephrotoxicity of antitumor drugs, which is in line with the data of other scientists [6,7,9,11]. Enzymuria may depend on individual features of drug metabolism in the patients, specifically, on their probable accumulation in the plasma; this makes measurements of urinary enzymes more valuable for preclinical diagnosis of nephrotoxicity.

Variability of enzymuria characteristic of membrane-bound enzymes first of all makes us doubt the rightfulness of using only normal values for individual assessment of the toxic effects of drugs on the patient's kidneys even if reference values are defined for every laboratory. Therefore, the activities of urinary enzymes should be measured before treatment, so that the results can be used as the initial value for comparing with the values in the course of treatment and assessing the degree of hyperenzymuria.

Thus, an increase in the activities of urinary enzymes is an early and the most sensitive indicator of nephrotoxicity of antitumor drugs. Many-fold (more than 10-20 times) increase in enzymuria in comparison with the initial level or combined increase of the urinary enzymes activities and of serum creatinine are factors of pronounced nephrotoxicity, which requires urgent detoxication measures or discontinuation of the drug.

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