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PROXIMAL TUBULE DAMAGE IN PATIENTS TREATED WITH GENTAMICIN OR AMIKACIN

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The present study aimed to determine the relationships between time of administration and dose of aminoglycosides, the extent of proximal tubule damage, evaluated by the urine N-acetyl-β-D-glucosaminidase (NAG) activity, and to compare proximal tubule dysfunction in the patients treated with gentamicin and those receiving amikacin. The measurement of activity of NAG in urine was chosen to monitor of proximal tubule function.

The studies were performed in 25 patients, who had to be administered gentamicin or amikacin by intramuscular injections. In both groups, the maximum NAG activities in urine were detected most frequently after the 7th day of the therapy. A significant difference in NAG activities in urine was noted between the values observed in the course of treatment with aminoglycosides and those determined before start of the treatment.

NAG activity in urine significantly decreased following discontinuation of aminoglycoside antibiotic administration. The activities did not decrease quite to the pretreatment level but the remaining difference proved to be insignificant. In the course of aminoglycoside treatment, 7 patients demonstrated an increase in serum creatinine levels exceeding 0.4 mg%. It should be stressed that no pronounced differences in nephrotoxicity and, in particular, in their potential to induce injury to the proximal tubule have been disclosed between gentamicin and amikacin. Their significant, damaging effect on integrity of proximal tubule was demonstrated, which was evidenced by the clear increase in urinary NAG activity during administration of either drug. Nevertheless, only in a small fraction of such cases (12–16%), the increase promoted development of renal insufficiency, usually of a transient character. Monitoring of the increase in urinary NAG activities in line with observations on creatinine levels permits to distinguish a subgroup of patients who may be suspected of development of overt nephrotoxicity. In such cases cessation of aminoglycoside administration is required.

Key words: *gentamicin, amikacin, tubular dysfunction, N-acetyl-β-D-glucosaminidase (NAG) urinary excretion*

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INTRODUCTION

Aminoglycosides are antibiotics which are widely applied in everyday clinical practice. On the other hand, they represent also a frequent cause of drug-induced acute non-inflammatory renal insufficiency (ARI). Nephrotoxic activity of the drugs is linked to their accumulation in renal cortex, dependent upon affinity of a drug to kidneys and on kinetics of drug trapping processes. A capacity of the drug to bind the phospholipids and to induce intracellular lesions (the so called intracellular toxic activity) are also significant determinants of nephrotoxicity.

The most frequently applied drugs of aminoglycoside group include gentamicin and amikacin. As compared to amikacin, gentamicin undergoes more extensive accumulation in kidneys, and the two antibiotics are trapped by cells of renal cortex *via* different mechanisms. A slight increase in gentamicin concentration is accompanied by a significant, incommensurate increase in the amount of the drug bound to renal cortex. On the other hand, binding of amikacin to renal cells is characterized by a linear relationship: rising serum concentrations of the drug is accompanied by a proportional increase in the bound drug [1]. With regard to intracellular toxicity, dependent on the number of free amine groups, gentamicin and amikacin exhibit similar profile.

Studies on animals suggest that amikacin induces signs of nephrotoxicity less frequently as compared to gentamicin, netylmycin or tobramycin [1, 7]. However, the data indicating more frequent nephrotoxicity in the patients treated with gentamicin than in those treated with amikacin are not fully unequivocal [16]. Capacity of a drug to evoke nephrotoxic effects is determined by several variables, including duration of drug administration, its dose, original status of renal function, frequency of administration, coexisting diseases and drugs administered in parallel, gender of the patient. An increase in serum creatinine level indicates the already overt renal damage but fails to provide data on the current extent of subclinical damage to proximal tubule cells.

A much more precise monitoring of proximal tubule function takes advantage of assays of some urinary enzymes, which are indicative of the early renal injury. Determination of activity of N-acetyl-β-D-glucosaminidase (NAG) in urine is the most appropriate marker of such injury.

The aims of the present studies can be defined as follows:

1. Determination of the relationships between time of administration and dose of aminoglycosides, and the extent of proximal tubule damage by evaluation of the urine NAG activity;
2. Comparison of proximal tubule dysfunction between patients treated with gentamicin and those treated with amikacin;
3. Determination of prognostic factors which would permit to predict nephrotoxicity in the course of aminoglycoside treatment.

PATIENTS and METHODS

Patients

The studies were performed in 25 patients (12 women and 13 men) hospitalized in the Ward of Internal Diseases and Rheumatology, Regional Railway Hospital in Wrocław due to infections, mainly infections of respiratory tract, who had to be administered antibiotics of aminoglycoside groups, gentamicin or amikacin, by intramuscular injections. The applied dose, frequency of administration, type of antibiotic and duration of treatment were adjusted according to condition of the patients and type of therapeutic reaction. Median age of the patients was 62 years (ranging from 22 to 85 years). Creatinine level was repeatedly estimated in serum. Control group consisted of 33 patients, 30 to 71 years of age, in whom hospital observation disclosed no alterations in the urinary system, who reported no disuric complaints and took no potentially nephrotoxic drugs.

Methods

NAG activity was estimated in the middle-stream morning urine [17, 20]. The technique of Merle et al. [10] was applied, with the slight modification introduced by Price et al. [12]. A sample of 50 μl of twenty fold-diluted urine was mixed with 950 μl of substrate solution (4-methylumbelliferyl-N-acetyl-β-D-glucosaminide – MUNAG; Sigma Chemical Co., St Louis, MO, USA) in 0.2 mM citrate-phosphate buffer, pH 4.74. After 30 min of incubation at 37°C, the reaction was stopped by the addition of 3 ml of 0.25 M glycine sodium salt solution, pH 10.5. The amount of released in the reac-

tion 4-methylumbelliferone anion was estimated by the measurement of an increase in fluorescence using spectrofluorimeter at the wavelength of 450 nm.

NAG activity was expressed in nanomoles (10^{-9} M) of 4-methylumbelliferone produced by the enzymatic reaction per 1 ml of urine per an hour. In order to avoid diurnal urine collection, the values were related to the current creatinine concentration in urine [17].

Statistical analysis

Significance of differences between subgroups was tested using non-parametric tests: the χ^2 test with Yates correction, U-test Mann-Whitney for two independent random samples and Wilcoxon's pair sequence test for two dependent samples. The differences were regarded significant if $p < 0.05$.

RESULTS

NAG activity in urine of control individuals amounted to 26.8 ± 17.7 (range: 3.4–71.3; median value: 24.1) nmol/mg of creatinine. The values of NAG activity within 95% confidence limits did not exceed 70 nmol/mg of creatinine and this value was accepted as the upper limit of the normal range.

Characteristics of the patients subjected to aminoglycoside treatment are presented in Table 1. The mean values discussed below were expressed as median values and the range of values was presented in brackets. On the average, the patients received a total of 1580 (800–2880) mg of gentamicin for the period of 10 (7 to 14) days. On the other hand, amikacin was administered at the average total dose of 7.5 (5.5 to 11) g for the period of 10 (6 to 14) days. NAG activity was estimated in each patient before the treatment with gentamicin or amikacin (n = 25), several times in the course of the treatment with the aminoglycosides (between the first and third day, fourth and sixth day and following the 7th day of the therapy, n = 131), and after termination of the therapy (n = 79). Before start of the therapy, most of the patients demonstrated abnormal NAG activities. This was noted in 85% of the patients before treatment with gentamicin (mean of 126.2 nmol/mg of creatinine), and in 100% patients before therapy with amikacin (mean of 161.6 nmol/mg of creatinine). No significant differences in urine NAG activity were detected between the patients treated with gentamicin and those treated with amikacin before aminoglycoside treatment, at individual time points during the treatment and after its discontinuation (Tab. 2). In the course of gentamicin treatment, 60 out of 72 (83%) tests yielded elevated activities of NAG (> 70 nmol/mg of creatinine) while in the patients treated with amikacin, 55 out of 59 (93%) NAG activities were abnormal. In both groups, maximum NAG activities in urine were detected most frequently after the 7th day of therapy. Median value of peak urine NAG activities in the patients treated with gentamicin was 832.4 (52.7–3350.6) nmol/mg of creatinine and resembled respective activities in the patients treated with amikacin: 751.1 (154.1–3425.2) nmol/mg creatinine. The peak values were 6.6 times higher than NAG activities observed at the start of gentamicin treatment and 4.7 times higher than NAG activities at the start of amikacin treatment. No significant differences between the two treatment groups could be observed when NAG activities at a given period of treatment were compared with the pretreatment NAG activities except that mean NAG activity in urine in the patients treated with amikacin after termination of the treatment was similar to that before the treatment while after termination of gentamicin administration, mean NAG activity in urine was still twice as high as before the treatment (Tab. 2).

A significant difference in urine NAG activities was noted between the values observed in the course of treatment with aminoglycosides and those determined before start of the treatment (NAG 0) (NAG activity on days 1 to 3 vs. NAG 0, $p < 0.05$; NAG

Table 1. Clinical data of the patients treated with gentamicin and amikacin

The reason of therapy with aminoglycosides:	
— pneumonia:	
a) gentamicin	18 (75%)
b) amikacin	11 (79%)
— urinary upper tract infection	7 (64%)
a) gentamicin	5 (20%)
b) amikacin	2 (14%)
— other: bursitis, thrombophlebitis	3 (27%)
a) gentamicin	2 (8%)
b) amikacin	2 (18%)

Table 2. NAG-enzymuria during treatment with gentamicin and amikacin [median]

	Total	Gentamicin	Amikacin	p
Number	25	14	11	
NAG-enzymuria before treatment (NAG 0)	137.4	126.2	161.6	NS
Median of NAG activities during therapy	410.8 (3.0)	399.5 (3.2)	466.7 (2.9)	NS
NAG^{max}	759 (5.0)	832. (6.6)	751.1 (4.7)	
Date of occurrence of NAG^{max}	7	7	7	
Median of NAG activities on day 1–3 of treatment	249.2 (1.8)	226.9 (1.8)	328.9 (2.0)	NS
Median of NAG activities on day 4–6 of treatment	399 (2.9)	313.8 (2.5)	452.5 (2.8)	NS
Median of NAG activities after 7 days of treatment	691.2 (5.0)	517.6 (4.1)	786.4 (4.9)	NS
Median of NAG activities after treatment	206.4 (1.5)	237.8 (1.9)	168.6 (1.1)	NS

NS – not statistically significant. The ratios of NAG activity at the time point specified in the table to NAG activity before the treatment are reported in brackets. NAG^{max} – maximal NAG activity in the course of treatment with aminoglycosides

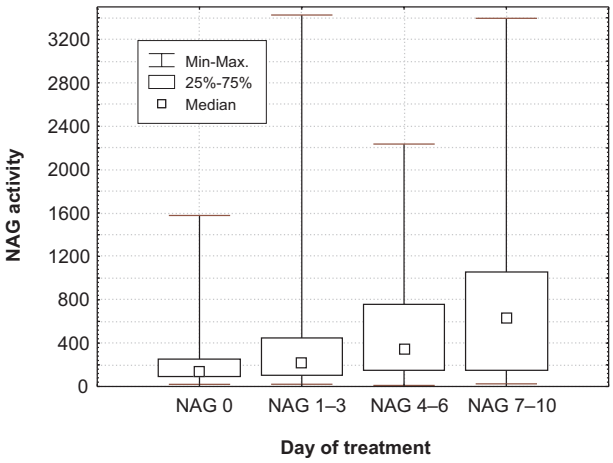

Fig. 1. NAG in the course of treatment of aminoglycosydes

activity on days 4 to 6 vs. NAG 0, $p < 0.005$, NAG activity following 7th day of the therapy vs. NAG 0, $p < 0.0005$) as well as between NAG activities in urine on days 1 to 3 on the one hand and the activities following the 7th day of treatment with aminoglycosides on the other (Fig. 1, $p < 0.005$, evaluated by the Wilcoxon's pair sequence test, for the numerical values see Table 2).

NAG activity in urine significantly decreased following discontinuation of aminoglycoside antibiotic administration, as demonstrated by the comparison of the median value of all NAG activities in the course of the treatment and the activity measured after termination of the treatment (410.8 vs. 206.4 nmol/mg of creatinine in the Wilcoxon's pair sequence test). The activities did not decrease exactly to the pretreatment level but the remaining difference proved to be insignificant (206.4 vs. 137.4 nmol/mg of creatinine, $p > 0.25$).

In the course of aminoglycoside treatment, 7 patients demonstrated an increase in serum creatinine levels exceeding 0.4 mg%, which, as suggested by Smith et al. [16], was taken as a criterion of nephrotoxicity. Three of these patients (12%) were treated with gentamicin and 4 (16%) with amikacin. In five of those 7 patients, maximum creatinine level exceeded upper limit of the norm 1.3 mg%. Due to low number of the patients, no prognostic factors were analyzed, which would indicate development of renal insufficiency. However, it could be noted that baseline levels of NAG-enzymuria were similar in patients with elevated serum creatinine levels and in patients without such elevation although patients with nephrotoxicity manifested faster increase in urine NAG activities (Tab. 3). In the patients with baseline urine NAG activities higher than double upper limit of their norm (140 nmol/mg of creatinine) serum creatinine values did not significantly exceed the values noted in the patients with baseline NAG activities lower than 140 nmol/mg of creatinine. In fact, the latter group of the patients demonstrated faster increase in urine NAG activities and higher creatinine levels (with peak creatinine value of 1.12 ± 0.48 vs. 1.38 ± 0.7 mg% and median value of ratio NAG activity in the course of treatment to NAG before treatment: 1.57 vs. 3.0). No correlation could also be detected between cumulative dose of gentamicin or amikacin

Table 3. Comparison of the patients with and without nephrotoxicity after aminoglycoside treatment

	Nephrotoxicity	No nephrotoxicity
Age (years)	64.9 ± 11.2	58.5 ± 16.4
Creatinine before treatment	0.98 ± 0.27 mg% (87 ± 24 μmol/l)	0.85 ± 0.26 mg% (75 ± 23 μmol/l)
Maximal levels of creatinine	1.97 ± 0.7 mg% (174 ± 62 μmol/l)	0.97 ± 0.22 mg% (86 ± 19 μmol/l)
NAG-enzymuria before treatment (NAG 0)	161.6	136.5
Median of NAG activities during therapy	496.4 (3.1)	366.9 (2.7)
NAG^{max}	973.5 (6.0)	755.0 (5.5)
Median of NAG activities on day 1–3 of treatment	397.1 (2.5)	237.7 (1.7)
Median of NAG activities on day 4–6 of treatment	631.4 (3.9)	329.6 (2.4)
Median of NAG activities after 7 days of treatment	842.7 (5.2)	631.4 (4.6)
Median of NAG activities after treatment	325.4 (2.0)	204.5 (1.8)

NAG activity in urine is expressed in nmol/mg of creatinine as median. The ratios of NAG activity at the time point specified in the table to NAG activity before the treatment are reported in brackets

and urine NAG values (using Pearson's correlation coefficient).

DISCUSSION

From the morphological perspective, aminoglycosides bind to cell membranes of proximal tubules, wherefrom the drug is transported to interior of the cells by pinocytosis. Subsequently, it selectively binds to negatively charged lysosomes, forming the so-called myelin bodies. Swelling of the latter leads to rupture of lysosomes and to necrosis of the cells. For this reason, NAG, which is located first of all in lysosomes, represents a model parameter which permits to monitor proximal tubule damage in the course of treatment with aminoglycosides [2, 6, 9, 13, 21]**.** Monitoring of NAG-enzymuria allows to select a scheme of treatment which is most safe for renal function, particularly in cases when treatment with one or two antibiotics of aminoglycoside, cephalosporin or synthetic penicillin groups is indispensable [3, 5, 7, 8, 11, 16].

The frequently encountered problem in determining nephrotoxicity due to aminoglycoside treatment involves the difficulty in distinguishing the complication from other, coexisting conditions, which impair renal function, such as infection in urinary system and administration of other, potentially nephrotoxic drugs. Markedly elevated NAG activities in urine are encountered first of all in the damage to proximal tubules. This permits to distinguish the injury from the exclusively glomerular pathology, in which urinary NAG activity is normal or only moderately elevated. Obviously, in general clinical practice, an impaired renal function is frequently of a mixed nature, including both glomerular and proximal tubule pathology. In our studies, in most of the patients the elevated NAG activities in urine have been observed already before start of the aminoglycoside treatment. A majority of the patients were treated for pneumonia. General infections could also induce reactive tubular-interstitial lesions in the kidneys or microorganisms might directly affect the proximal tubule [4].

Aminoglycosides accumulate in all tissues. At first, they bind to cell membranes and, then, they slowly penetrate cell interior. Half-time of the process ranges from 7 to 70 h. Morphological alterations detected in animals subjected to aminoglycoside action resemble abnormalities observed in the patients administered with aminoglycosides. In the course of prolonged aminoglycoside administration, their concentration in kidneys slowly increases. McCluskey et al. evaluated changes in tubular cells of Lewis rats and noted most pronounced lesions between 7th and 10th day after start of the therapy. In the subsequent days, reparatory processes were noted even if aminoglycoside

administration was continued [19]. Whiting and Brown monitored NAG activities in animals employing gentamicin-induced nephrotoxicity model and demonstrated that the activity returned to normal levels on day 14 of administering the drug [22].

Our results, demonstrating the most pronounced injury to the proximal tubule following the 7th day of the therapy, are consistent with results of experimental studies although, for the reason of safety, we have seldom administered the antibiotics for a period longer than 10 days. Similar observations have been provided by clinical studies of Gibey et al. on gentamicin-treated patients [6].

In our studies, the studied patients were hospitalized and represented a subgroup of seriously ill patients. The frequency of nephrotoxicity which we have observed in our patients (12% for gentamicin and 16% for amikacin) resembles the data obtained by Smith et al. and by French et al. on groups of patients similar in respect to clinical condition [5, 16]. In the patients treated with gentamicin or amikacin, the abovementioned authors recorded nephrotoxicity in 11 and 16% as well as 8 and 20% patients, respectively. The cited authors also have found no differences in pharmacokinetics between the two drugs or their accumulation in renal tissue. Results of some studies have indicated that frequency of aminoglycoside nephrotoxicity decreases when drugs of the group are given once daily as compared to the situation when they are given several times a day [1, 14], although the data have occasionally been questioned [24]. Patients of our group used to receive gentamicin 2–3 times a day for the first 3 days and once a day in subsequent days.

According to Gibey et al. and Donta and Lembke, the preliminary markedly elevated urinary NAG activity may be of prognostic significance for the subsequent development of nephrotoxicity [3, 6], which has not been confirmed by results of our study. A similar absence of relationships between preliminary levels of β-2-microglobulin (early marker of proximal tubule injury) and subsequently elevated creatinine levels has been described by Schentag and Plaut in patients treated with aminoglycosides [15].

The observed by us ratio of urinary NAG activities in the course of treatment to those noted before the treatment has resembled the data obtained by Wellwood et al. in the patients treated with gentamicin after transplantation [21]. A significant increase in NAG activities in the course of aminoglycoside treatment points to their damaging effect on proximal tubule cells but it is not automatically indicative of less efficient renal function, indicated by augmented creatinine levels. Estimation of markers of early nephrotoxicity, e.g. NAG in urine or of β-2-microglobulin, permits to monitor proximal tubule injury which is most frequently transient and only in some cases may lead to significant nephrotoxicity [8].

Nevertheless, a rapid increase in urinary NAG activity may indicate potential for development of renal insufficiency, which has been demonstrated also in the patients treated with two potentially nephrotoxic drugs or with the already existing renal insufficiency [18, 23].

Summing up, it should be stressed that no pronounced differences, have been disclosed between gentamicin and amikacin in nephrotoxicity and, in particular, in their potential to induce injury to the proximal tubule. Their significant, damaging effect on integrity of proximal tubule was demonstrated, which was evidenced by the evident increase in urinary NAG activity during administration of either drug. Nevertheless, only in a small fraction of such cases (12–16%) the increase promoted development of renal insufficiency, usually of a transient character. Monitoring of the increase in urinary NAG activities concomitantly with observations on creatinine levels permits to distinguish a subgroup of patients in whom development of overt nephrotoxicity may be suspected. In such cases cessation of aminoglycoside administration is required.

REFERENCES

1. De Broe M.E., Giuliano R.A., Verpooten G.A.: Choice of drug and dosage regimen. Two important risk factors for aminoglycoside nephrotoxicity. Amer. J. Med., 1986, 80, 115–118.
2. Diener U., Knoll E., Langer B., Rautenstrauch H., Ratge D., Wisser H.: Urinary excretion of N-acetyl-beta-D-glucosaminidase and alanine aminopeptidase in patients receiving amikacin or cis-platinum. Clin. Chim. Acta, 1981, 112, 149–157.
3. Donta S.T., Lembke S.A.: Comparative effects of gentamicin and tobramycin on excretion of N-acetyl-beta-D-glucosaminidase. Antimicrob. Agents Chemother., 1985, 28, 500–503.
4. Ellis D., Fried W.A., Yunis E.J., Blau E.B.: Acute interstitial nephritis in children. A report of 13 cases and review of the literature. Pediatrics, 1981, 67, 862–870.
5. French M.A., Cerra F.B., Plaut M.E., Schentag J.J.: Amikacin and gentamicin accumulation pharmacoki-

netics and nephrotoxicity in critically ill patients. Antimicrob. Agents. Chemother., 1981, 19, 147–152.
6. Gibey R., Dupond J.L., Alber D., Leconte des Floris R., Henry J.C.: Predictive value of urinary N-acetyl-beta-D-glucosaminidase, alanine-aminopeptidase and beta 2-microglobulin in evaluating nephrotoxicity of gentamicin. Clin. Chim. Acta, 1981, 116, 25–34.
7. Gibey R., Mozer J.L., Henry J.C.: Nephrotoxicity of the combination of cyclosporin and aminoglycoside in rats. Comparative study of gentamicin and amikacin. Pathol. Biol., 1990, 38, 513–516.
8. Kepczyk T., Ryan P.J. 3rd, McAllister K., Otraje J.: The absence of nephrotoxicity and differential nephrotoxicity between tobramycin and gentamicin. South Med. J., 1990, 83, 1149–1152.
9. Marchewka Z., Długosz A.: Enzymes in urine as markers of nephrotoxicity of cytostatic agents and aminoglycoside antibiotics. Int. Urol. Nephrol., 1998, 30, 339–348.
10. Merle L.J., Reidenberg M.M., Camacho M.T.: Renal injury in patients with rheumatoid arthritis treated with gold. Clin. Pharmacol. Ther., 1980, 27, 557–562.
11. Nix D.E., Thomas J.K., Symonds W.T., Spivey J.M., Wilton J.H., Gagliar N.C., Schentag J.J.: Assessment of the enzymuria resulting from gentamicin alone and combinations of gentamicin with various beta-lactam antibiotics. Ann. Pharmacother., 1997, 31, 696–703.
12. Price R.G., Dance N., Richards B., Cattell W.R.: The excretion of N-acetyl-beta-D-glucosaminidase and beta-galactosidase following surgery to the kidney. Clin. Chim. Acta, 1970, 27, 67–72.
13. Ring E., Eber E., Erwa W., Zachs M.S.: Urinary N-acetyl-beta-D-glucosaminidase activity in patients with cystic fibrosis on long-term gentamicin inhalation. Arch. Dis. Child., 1998, 78, 540–543.
14. Rybak M.J., Abate B.J., Kang S.L., Ruffing M.J., Lerner S.A., Drusano G.L.: Prospective evaluation of the effect of an aminoglycoside dosing regimen on rates of observed nephrotoxicity and ototoxicity. Antimicrob. Agents Chemother., 1999, 43, 1549–1555.
15. Schentag J.J., Plaut M.E.: Patterns of urinary beta 2-microglobulin excretion by patients treated with aminoglycosides. Kidney Int., 1980, 17, 654–661.
16. Smith C.R., Baughman K.L., Edwards C.Q., Rogers J.F., Lietman P.S.: Controlled comparison of amikacin and gentamicin. N. Engl. J. Med., 1977, 296, 349–353.
17. Stolarek J., Howey J.E.A., Fraser C.G.: Biological variations of urinary N-acetyl-beta-D-glucosaminidase: practical clinical implications. Clin. Chim. Acta, 1989, 35, 560–563.
18. Szechiński J., Wiland P.: Renal proximal dysfunction based on activity of N-acetyl-beta-D-glucosaminidase in urine of patients with kidney failure (Polish). Pol. Arch. Med. Wewn., 1997, 98, 534–541.
19. Tolkoff-Rubin N.E., Haddad E.P., McCluskey R.T., Bhan A.K., Rubin R.H.: Renal damage due to aminoglycoside administration in the Lewis rat. In: Acute Renal Failure. Eds. Solez K., Whelton A., Marcel Dekker, New York, 1984, 249–259.
20. Wellwood J.M., Price R.G., Ellis B.G., Thompson A.E.: A note on the practical aspects of the assay of N-acetyl-β-glucosaminidase in human urine. Clin. Chim. Acta, 1976, 69, 85–91.
21. Wellwood J.M., Simpson P.M., Tighe J.R.: Evidence of gentamicin nephrotoxicity in patients with renal allografts. Brit. Med. J., 1975, 3, 278–281.
22. Whiting P.H., Brown P.A.: The relationship between enzymuria and kidney enzyme activities in experimental gentamicin nephrotoxicity. Renal Fail., 1996, 18, 899–909.
23. Wiland P., Szechiński J.: N-acetyl-beta-D-glucosaminidase enzymuria as an indicator in monitoring the therapy of some rheumatic diseases with potentially nephrotoxic drugs. Arch. Immunol. Ther. Exp., 1994, 42, 331–336.
24. Zhanel G.G., Ariano R.E.: Once daily aminoglycoside dosing: maintained efficacy with reduced nephrotoxicity? Renal Fail., 1992, 14, 1–9.

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