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ACTIVITIES OF PROXIMAL TUBULE ENZYMES IN URINE OF PATIENTS TREATED WITH GENTAMICIN

AKTIVNOST ENZIMA PROKSIMALNIH TUBULA U URINU PACIJENATA TRETIRANIH GENTAMICINOM

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Summary: The activities of the enzymes dominantly localized within the proximal tubules, such as alanine aminopeptidase (AAP), gamma-glutamyl transferase (GGT) and N-acetyl-beta-D-glucosaminidase (NAG), were measured in 12-h urine samples of patients suffering from Gram-negative infections and i.v. treated with gentamicin with the aim of determining the nephrotoxicity of this aminoglycoside antibiotic. The examined groups consisted of 3–10 years old children of both sexes, gentamicin-treated, and the control group, each including 30 patients. Urine samples were collected and analyzed five days before the gentamicin application and during the following 10 days of gentamicin treatment (a single i.v. injection per day in the dose of 2.5 mg/kg b.w.). Significant differences in the AAP and GGT activities expressed in U/mmol creatinine were observed between the gentamicin-treated group and the controls already on day 2 ($p < 0.05$) of the treatment, as well as in the activity of NAG on day 8 ($p < 0.01$) of the therapy. From these results it can be concluded that even standard gentamicin doses expressed nephrotoxic effects. Statistically significantly increased AAP and GGT activities in the gentamicin-treated group of children recorded already on the 2nd day of treatment demonstrate that these two enzymes represent extremely sensitive indicators of nephrotoxicity. On the other hand, statistically significantly increased NAG activity observed in the gentamicin-receiving group points to more severe gentamicin-provoked injuries of proximal tubules.

Keywords: alanine aminopeptidase (AAP), gamma-glutamyl transferase (GGT), N-acetyl-beta-D-glucosaminidase (NAG), urine, gentamicin, nephrotoxicity

Kratak sadržaj: Radi utvrđivanja nefrotoksičnosti aminoglikozidnog antibiotika gentamicina, određivana je aktivnost enzima dominantno lociranih u proksimalnim tubulama: alanin aminopeptidaze (AAP), gama-glutamiltransferaze (GGT) i N-acetil-beta-D-glukozaminidaze (NAG), u uzorcima 12-časovnog urina u 30 ispitanika kojima je, zbog gram-negativnih infekcija, intravenski apliciran gentamicin. Aktivnost istih enzima određivana je i u 12-časovnom urinu 30 ispitanika kontrolne grupe. Polnu strukturu grupa činili su ispitanici oba pola, starosti od 3 do 10 godina. Predtretman, sa obe grupe i bez apliciranja gentamicina, vođen je 5 dana, a tretman u periodu od 10 dana, pri čemu su ispitanici eksperimentalne grupe primali jednokratno gentamicin u dozama od 2,5 mg/kg tjelesne mase dnevno. Značajne razlike u aktivnostima AAP i GGT, u U/mmol kreatinina, utvrđene su između eksperimentalne i kontrolne grupe drugog dana ($p<0,05$), i u aktivnostima NAG osmog dana ($p<0,01$) sprovođenja terapije. Može se zaključiti da desetodnevni tretman gentamicinom, čak i sa normalnim dozama, uslovljava nefrotoksične efekte. Pri tom su statistički značajno povišene vrijednosti AAP i GGT ranih i ekstremno osjetljivih indikatora nefrotoksičnosti, dok je statistički značajno povišenje aktivnosti NAG dijagnostički znak gentamicinom izazvanih težih oštećenja proksimalnih tubula.

Ključne riječi: alanin aminopeptidaza (AAP), gama-glutamil transferaza (GGT), N-acetil-beta-D-glukozaminidaza (NAG), urin, gentamicin, nefrotoksičnost

Introduction

Aminoglycoside antibiotics play a leading role in the therapy of different Gram-negative infections during which nephrotoxic complications were recorded in 10–15% of cases (1). Secondary nephrotoxicity of gentamicin was shown to be related to the action of free oxygen radicals (2), and it is accompanied by

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increased activities of AAP (EC 3.4.11.2), GGT (EC 2.3.2.2) and NAG (EC 3.2.1.30) in urine, all these enzymes being dominantly localized in the proximal tubules (3).

Numerous authors clearly demonstrated the nephrotoxic effects of high gentamicin doses applied to experimental animals by the determination of AAP, GGT and NAG activites in urine (4–10). However, the results of clinical studies performed on patients receiving a standard gentamicin dose of 2.5 mg/kg b.w. for 10 days, aimed at the examination of whether the changes in activity of the above-mentioned enzymes in urine could serve as reliable markers of gentamicin-related early injuries of proximal tubules, were somewhat contradictory (11–13). This prompted us to examine the activity of AAP, GGT and NAG in urine samples of patients treated for 10 days with a standard gentamicin dose of 2.5 mg/kg b.w. and to find out whether these enzymes could serve as markers of early proximal tubule injuries.

Materials and Methods

The examinations included sixty 3–10 years old children of both sexes, patients of the Pediatric Clinic of the Clinical Centre in Banjaluka. Thirty children were treated with a single i.v. injection of gentamicin, in the dose of 2.5 mg/kg b.w./day during 10 consecutive days, against Gram-negative bacterial infections. A group of 30 age- and sex structure-matched children without health problems with the urogenital tract served as controls.

Morning 12-h urine was collected and kept at –25 °C till the analyses of enzyme activities. Urine collection started 5 days before the onset of gentamicin treatment. The data on the age, sex and state of health of the patients were obtained by a questionnaire on the first day of the collection of urine samples.

In order to separate the enzymes, urine samples were subjected to gel filtration (14). The activities of AAP (15), GGT (16, 17) and NAG (18), as well as creatinine concentrations (19), were determined by photometry. Enzyme activities were expressed as U/mmol creatinine.

The results were analyzed by standard statistical methods and expressed as means ±S.D. Significance of the differences between the groups was evaluated by Student's t-test. Statistical significance was accepted at $p<0.05$.

Results

Mean values of AAP, GGT and NAG activities, expressed in U/mmol creatinine, are depicted in *Figures 1*, *2* and *3*, respectively. During the 5-day pretreatment period, variations in the activity of exami-

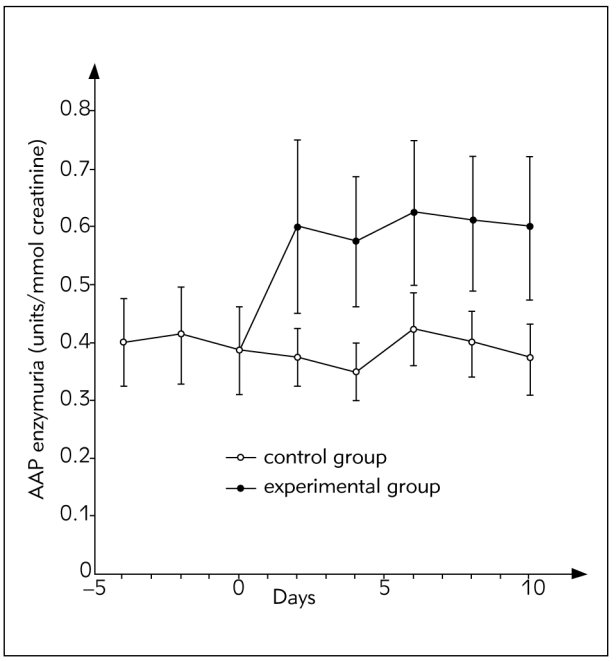

Figure 1 Enzymatic activity of AAP in urine of children receiving a single gentamicin injection in the dose of 2.5 mg/kg b.w./day i.v., 4 and 2 days before gentamicin application and on days 2, 4, 6, 8 and 10 of therapy, and in urine of the corresponding controls. Results are expressed as means ±S.D.

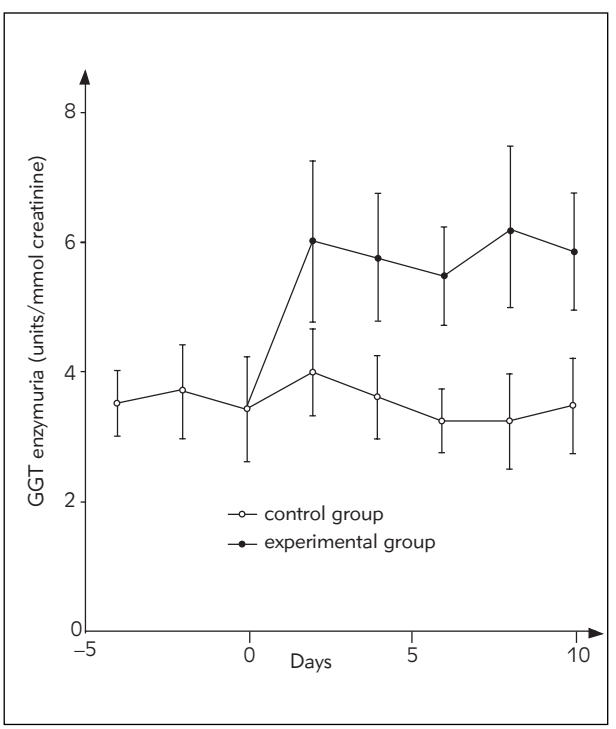

Figure 2 Enzymatic activity of GGT in urine of children treated with a single gentamicin injection in the dose of 2.5 mg/kg b.w./day by i.v. route, 4 and 2 days before the onset of gentamicin therapy and on days 2, 4, 6, 8 and 10 of therapy, and in urine of the corresponding controls. Results are expressed as means ±S.D.

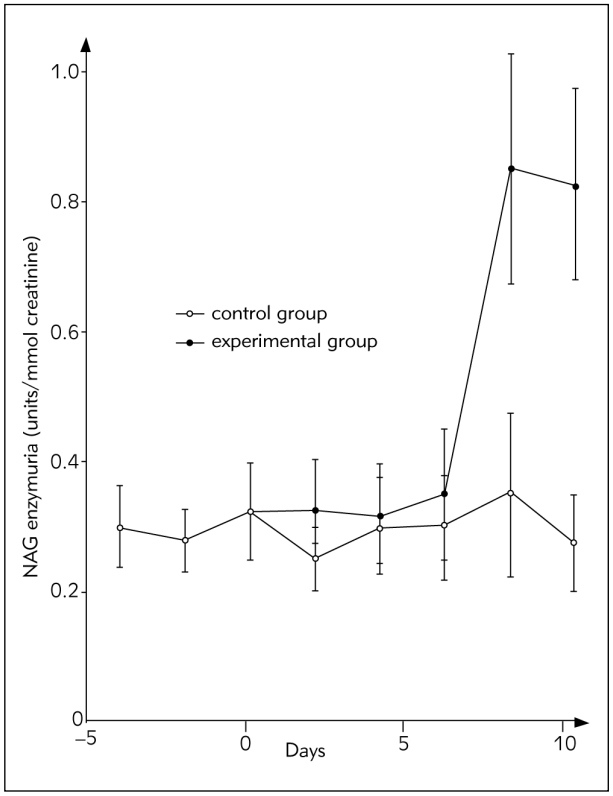

Figure 3 Enzymatic activity of NAG in urine of children receiving i.v. a single gentamicin injection in the dose of 2.5 mg/kg b.w./day, 4 and 2 days before gentamicin treatment and on days 2, 4, 6, 8 and 10 of therapy, and in urine of the corresponding controls. Results are expressed as means ±S.D.

ned enzymes of 4.0% (AAP), 4.8% (GGT) and 8.3% (NAG), were negligible.

Statistically significant increase of AAP activity ($\bar{x} = 0.60 \pm 0.15$), expressed as mean value ±S.D. in relation to that of the control group ($\bar{x} = 0.37 \pm 0.07$; $p < 0.05$) was recorded already on the second day after the onset of the therapy (*Figure 1*). During the following days of gentamicin treatment, increased AAP activity was observed, but the level of statistical significance of the differences between the gentamicin-treated group and the control one remained roughly the same.

The activity of GGT was also significantly increased in the gentamicin-treated group of patients in comparison with the control group ($\bar{x} = 6.0 \pm 1.3$ *vs.* $\bar{x} = 3.9 \pm 0.8$; $p<0.05$) on the second day of the therapy, and, similar to AAP, the level of statistical significance of the differences between the groups was roughly the same till the end of the antibiotic application (*Figure 2*).

From the data graphically presented in *Figure 3*, it can be seen that the activity of NAG was almost unchanged in the gentamicin-treated group in relation to controls during the first seven days of therapy. The increase in the activity of this enzyme was recorded on day 8 of the treatment and the mean values were significantly higher in the gentamicin-treated group in comparison with the controls ($\bar{x} = 0.85 \pm 0.19$ *vs.* $\bar{x} = 0.35 \pm 0.13$; $p<0.01$). The level of statistical significance between the groups remained constant during the last two days of gentamicin therapy.

Discussion

In the present study, the possible nephrotoxicity of gentamicin given in a single i.v. dose of 2.5 mg/kg/ /day to 3–10 years old children with Gram-negative infections was examined. The study was based on the results of Bennet (20), who reported that aminoglycoside nephrotoxicity is characterized by decreased glomerular filtration and necrosis of proximal tubules accompanied by increased activities of the enzymes localized within the proximal tubules which are excreted in urine. The examinations included 3–10 years old children, because, as shown previously, the excretion of enzymes from proximal tubules in infants is unstable and relatively lower values were obtained for the activity of the characteristic enzymes in urine, when compared to older children (21). The gentamicin-treated group consisted of children with Gram-negative infections, 40% of which suffered from urinary tract infections and 60% from pyelonephritis.

Our results confirmed the hypothesis that even if applied in normal doses, gentamicin expresses nephrotoxic effects at the cellular level, resulting in increased activities of several proximal tubule enzymes. The increase of brush border enzyme activities, i.e. AAP and GGT, was observed rather early, already on day 2 of the gentamicin therapy, while that of the NAG was recorded much later, on day 8 of this antibiotic application (*Figures 1–3*). This is in disaccord with the results of Ahijad and Garcia (22) who reported the appearance of the first nephrotoxic gentamicin-induced symptoms on day 7 of gentamicin therapy. After the first elevation of AAP and GGT activity observed in the present study, the level of significance of the differences between the gentamicin-treated group of children and the corresponding control remained roughly the same. This means that nephrotoxicity depends on individual gentamicin dose and not on the duration of therapy. Opposite to that, our results showed increased NAG activity close to the end of therapy, suggesting that it was more dependent on the duration of gentamicin treatment than on the dose applied. Similar to the data of Kos et al (13), the results presented here showed that increased activity of proximal tubule enzymes in urine was not accompanied by clinical signs of kidney injuries. Faster response of AAP and GGT during gentamicin application in comparison to that of NAG could be ascribed to the appearance of pathohistological changes of kidney tubule organelles at subcellular level at

the different time points of the therapy. It is also possible that different mechanisms are involved in the control of these enzyme activities. It is quite believable that two independent mechanisms play a role in gentamicin-related nephrotoxicity, i.e. that brush border cells of the proximal tubule epithelium are much more sensitive and respond much earlier to single daily gentamicin doses than the lysosomes of proximal tubule cells. Because of that, increased activities of AAP and GGT could be interpreted as the sign of less severe injury of proximal tubule cells, and these two enzymes could be taken as early and very sensitive indicators of gentamicin nephrotoxicity. Damages of lysosomes in proximal tubule cells result from the cumulative action of gentamicin, and this was the reason for the relatively late increase of NAG activity which can be considered as an indicator of more severe injuries of proximal tubule cells.

From the results obtained throughout the present study, it can be concluded that even normal gentamicin doses express nephrotoxic effects followed by elevated enzymatic activities of AAT, GGT and NAG, i.e. enzymes dominantly localized within the proximal tubules. Since both AAT and GGT responded to gentamicin treatment already on the 2nd day of the therapy, they are obviously very sensitive and reliable indicators of the nephrotoxicity of this aminoglycoside antibiotic. As shown here, NAG activity depends more on the duration of therapy than on the individual gentamicin doses applied, and increased activity of this enzyme indicates more serious damages of proximal tubule cells.

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