

URINARY ACTIVITIES OF PROXIMAL TUBULE ENZYMES IN NEONATES TREATED WITH GENTAMICIN

AKTIVNOSTI ENZIMA PROKSIMALNIH TUBULA U URINU PACIJENATA U NEONATALNOM PERIODU TRETIRANIH GENTAMICINOM

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Summary: In order to determine the nephrotoxicity of gentamicin, an aminoglycoside antibiotic, activity of the enzymes dominantly localized in proximal tubules, i.e. alanine aminopeptidase (AAP), γ -glutamyl transferase (GGT) and N-acetyl- β -D-glucosaminidase (NAG) was examined. Determinations were performed in 12-h urine samples of 30 neonates i.v. receiving gentamicin against Gram negative infections in daily doses of 5.0 mg/kg body mass for 10 consecutive days. The activities of the same enzymes were measured in 12-h urine samples of 30 examined of the control group. The groups consisted of neonates of both sexes. The pretreatment period lasted for 5 days. On day 8 of gentamicin application, statistically significant differences in the activity of AAP and GGT expressed in U/mmol creatinine between the gentamicin-receiving and control group ($p < 0.01$) were found. No significant differences in NAG activity of the gentamicin-treated group in comparison with the control were recorded during the 10-day gentamicin therapy. It can be concluded that 10-day treatment of neonates with usually prescribed gentamicin doses results in mild nephrotoxic changes close to the end of the therapy accompanied by increased activity of both urinary AAP and GGT, known as very sensitive indicators of nephrotoxicity. During the same treatment period no changes in NAG activity were observed, meaning that the antibiotic causes no severe injuries to proximal tubule cells at the level of cellular organelles.

Keywords: alanine aminopeptidase (AAP), γ -glutamyl transferase (GGT), N-acetyl- β -D-glucosaminidase (NAG), urine, gentamicin, neonates

Kratak sadržaj: Radi utvrđivanja nefrotoksičnosti aminoglikozidnog antibiotika gentamicina određivana je aktivnost enzima dominantno lokalizovanih u proksimalnim tubulama, alaninaminopeptidaze (AAP), γ -glutamyl-transferaze (GGT) i N-acetil- β -D-glukozaminidaze (NAG). Određivanje je vršeno u uzorcima 12-časovnog urina 30 ispitanika kojima je zbog Gram-negativnih infekcija intravenski apliciran gentamicin u dozama od 5,0 mg/kg telesne mase dnevno. Aktivnosti istih enzima su određivane i u 12-časovnom urinu 30 ispitanika kontrolne grupe. Polnu strukturu grupa su činili ispitanici oba pola i uzrasta neonatalnog perioda. Posle petodnevno perioda pre tretiranja, eksperimentalna grupa je dobijala gentamicin tokom 10 dana. Statistički značajne razlike u aktivnostima AAP i GGT, izražene u U/mmol kreatinina, utvrđene su između ispitanika eksperimentalne i ispitanika kontrolne grupe osmog dana ($p < 0,01$) sprovođenja terapije. Aktivnosti NAG kod ispitanika eksperimentalne grupe u odnosu na ispitanike kontrolne grupe se nisu značajno menjale tokom desetodnevno vremena sprovođenja terapije. Može se zaključiti da desetodnevni tretman ispitanika u neonatalnom periodu uobičajenim dozama gentamicina izaziva blage nefrotoksične promene koje su praćene porastom aktivnosti AAP i GGT, veoma osetljivih indikatora nefrotoksičnosti, tek pri kraju sprovođenja terapije. Za isto vreme sprovođenja terapije nije došlo do porasta aktivnosti NAG, što znači da nema težih oštećenja ćelija proksimalnih tubula na nivou ćelijskih organela.

Ključne reči: alaninaminopeptidaza (AAP), γ -glutamyl-transferaza (GGT), N-acetil- β -D-glukozaminidaza (NAG), urin, gentamicin, neonatalni period

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Introduction

Aminoglycoside antibiotics are mostly applied in bacterial therapy of Gram negative infections. In 10–15% cases their application leads to nephrotoxic changes, usually resulting from overdoses of the anti-

biotic, duration of the therapy or exceeding limits of individual doses (1). Nephrotoxicity of gentamicin, one of the most frequently used aminoglycoside antibiotics, originates from the action of free oxygen radicals evolving during its catabolism in human organisms (2). Its nephrotoxicity results in increased urinary activity of several enzymes dominantly localized in proximal tubules, such as AAP (EC 3.4.11.2), GGT (EC 2.3.2.2) and NAG (EC 3.2.1.30) (3). The results of numerous earlier studies on both experimental animals (4–7) and humans (8–13) confirmed that increased activity of the above enzymes in urine can be used as an early and sensitive indicator of gentamicin nephrotoxicity.

All investigations on the nephrotoxic action of gentamicin on humans published till present were performed on adults or children at the age when morphological and functional kidney formation has been completed. However, during the neonatal period, the process of kidney formation is not completed and neonates have less expressed renal accumulation of aminoglycoside antibiotics than the above groups of examinees. This prompted us to study changes in the enzymatic activity of AAP, GGT and NAG in urine samples of neonates treated with gentamicin against Gram negative infections, in comparison with those of elder children under identical conditions of the treatment.

Material and Methods

The investigations included 60 neonates of both sexes. The examinees were the patients of the Pediatric Clinic of the Clinical Center in Banjaluka. Experimental group consisted of 30 examinees treated with daily gentamicin doses of 2.5 mg/kg body mass by i.v. route against Gram negative infections during 10 consecutive days. The control group included the same number of examinees who had no health problems related to the urinary tract.

Morning 12-hour urine samples were collected and kept at -25°C until the analyses. Collecting of urine samples of all examinees in the experimental group started 5 days before gentamicin application (pretreatment period). The data on age, sex and health state of the examinees were introduced into a questionnaire at the onset of the first urine sample collection.

After the separation of the examined enzymes from urine by gel filtration (14), the levels of AAP (15), GGT (16, 17), NAG (18) and creatinine (19) were determined by photometric methods. Enzymatic activities were expressed in U/mmol creatinine.

The results analyzed by standard statistical methods and expressed as the means \pm S.D. were graphically presented. Statistical significance of the differences between the groups determined by Student *t*-test was accepted at $p < 0.01$.

Results

The means of enzymatic activities of AAP, GGT and NAG \pm S.D. expressed as U/mmol creatinine are presented in *Figure 1–3*. Variations in the activity of the examined enzymes during the 5-day pretreatment period were 4.4%, 5.1% and 6.6% for AAP, GGT and NAG, respectively.

Statistically significant increase of AAP activity in urine samples of the examinees treated with gentamicin, expressed as the mean \pm S.D. (0.57 ± 0.12), in relation to those of the control (0.34 ± 0.04) ($p < 0.01$), was recorded on day 8 after the onset of gentamicin application (*Figure 1*).

Similarly, the activities of GGT in urine samples of the examinees of the experimental group were also increased (5.6 ± 1.5) in comparison with those of the control group (3.6 ± 0.9 ; $p < 0.01$) on day 8 of gentamicin application and the difference was statistically significant (*Figure 2*).

Upon significant activity increase of both urinary AAP and GGT of gentamicin-treated patients recorded on day 8 of the therapy, statistical significance of the differences between the experimental and control group remained the same up to the end of gentamicin application.

The values of NAG activity in urine samples of experimental and control groups are depicted in *Figure 3*.

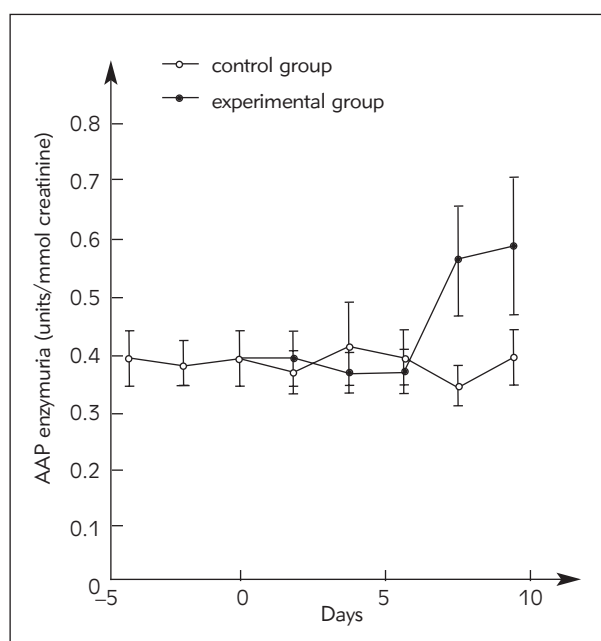


Figure 1 Mean values \pm S.D. of urinary AAP activity in neonates of the control and experimental group 4 and 2 days before gentamicin application and on day 2, 4, 6, 8 and 10 of gentamicin treatment.

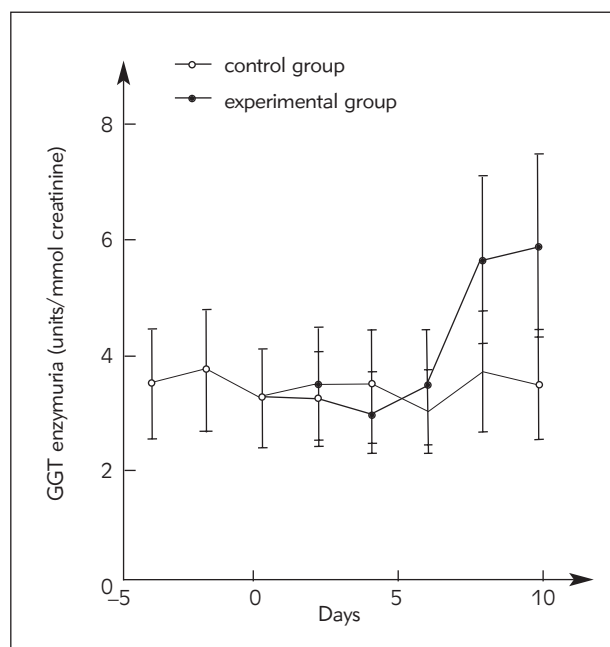


Figure 2 Mean values \pm S.D. of urinary GGT activity in neonates of the control and experimental group 4 and 2 days before gentamicin application and on day 2, 4, 6, 8 and 10 of gentamicin treatment.

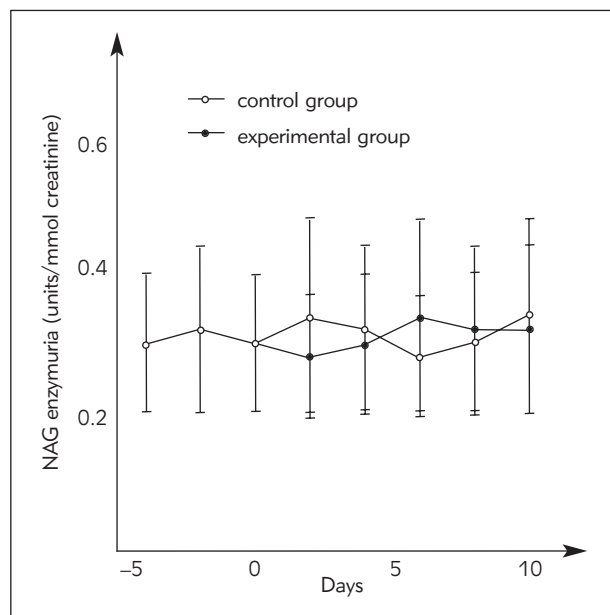


Figure 3 Mean values \pm S.D. of urinary NAG activity in neonates of the control and experimental group 4 and 2 days before gentamicin application and on day 2, 4, 6, 8 and 10 of gentamicin treatment.

It can be seen that gentamicin expressed no effect on the activity of this enzyme during the 10-day period, i.e. no statistically significant differences between the experimental and control group were recorded.

Discussion

The results of our earlier studies clearly demonstrated that even normal gentamicin doses given to the patients with completed morphological and functional kidney development, expressed nephrotoxic effects accompanied by increased activities of the enzymes dominantly localized in proximal tubule cells. Since in the children, examinees of neonatal period, the process of kidney formation is still progressing, while gentamicin accumulation in their renal tissue is lower, it was to be expected that the nephrotoxic action of this antibiotic should be less expressed than in older children and adults. Based on the results of his investigations, Heimann (20) pointed out to such a possibility.

In the present work, gentamicin was i.v. applied in usual individual daily doses of 5.0 mg/kg body mass to neonates suffering from Gram negative infections (urinary tract infections or pyelonephritis). Our results on the enzymatic activity of AAP, GGT and NAG found during the pretreatment period lasting for five days revealed no statistically significant differences between the experimental and control group. Significant elevation in the activity of brush border enzymes AAP and GGT was recorded only on day 8 of gentamicin application in examinees of the gentamicin-treated group comparing to the corresponding control (Figure 1 and 2). However, the results of our previous studies showed increased activities of the above two enzymes already on day 2 of gentamicin therapy in the group of 3–5 years old children (11, 21). The data of the present study corroborates these results demonstrating that gentamicin acts by injuring the cell membranes of proximal tubule epithelial cells in neonates, leading to increased enzymatic activities of urinary AAP and GGT localized in these membranes. However, these changes were recorded only on day 8 of gentamicin therapy, i.e. after a much longer treatment period than in 3–5 years old patients. This is in accordance with the reports of Alijadoa and Garcie (22), Simeoni et al. (23) and Heimann (20).

No significant increase in the NAG activity was observed during the 10-day gentamicin therapy in the urine samples of examinees of the experimental group in comparison with the corresponding control (Figure 3). In our previous studies on 3–5 years old patients, significantly increased NAG activity was observed on day 8 of the gentamicin treatment in relation to the control (11). Based on these findings, as well as on the results presented here, it can be concluded that the degree of gentamicin-induced injuries of renal epithelial cells depends not only on the duration of the treatment, but also on the time of the antibiotic retention in proximal tubules. Since gentamicin retention in the proximal tubule epithelium of neonates is obviously insufficient to lead to the damages of organelles in proximal tubule cells, the activity of a lysosomal enzyme NAG remained unaffected by gentamicin treatment.

The results obtained throughout this study lead to the conclusion that application of usual 10-day gentamicin therapy to neonates suffering from Gram negative urinary tract infections activates only AAP and GGT, the enzymes of proximal tubule epithelial cell membranes, known as very sensitive and reliable indicators of nephrotoxicity at the end of the antibiotic treatment. At the same time, no increases of lysosomal NAG activity were registered during the therapy, meaning that gentamicin did not act by injuring

severely proximal tubule cells at the level of cellular organelles. Thus, the 10-day gentamicin therapy of neonates can be considered as absolutely safe and could be even prolonged for several days to reach the level of nephrotoxic effects observed in 3–5 years old children treated with this antibiotic for 10 days.

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