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THE ACTIVITY OF PROXIMAL TUBULE ENZYMES IN THE URINE OF CEPHALEXIN-TREATED PATIENTS

AKTIVNOST ENZIMA PROKSIMALNIH TUBULA U URINU PACIJENATA TRETIRANIH CEFALEKSINOM

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Summary: The activities of alanine aminopeptidase (AAP), γ-glutamyltransferase (GGT) and N-acetyl-β-D-glucosaminidase (NAG), enzymes dominantly localised in the epithelial proximal tubule cells, were measured with an aim of determining the nephrotoxicity of a cephalosporin antibiotic cephalexin. Enzymatic activities were measured in the 12-h urine samples of patients receiving cephalexin orally for 15 days in daily doses of 50 mg/kg body mass against Gram-positive infections of the respiratory or urinary tract. The same enzymes were determined in the 12-h urine samples of the corresponding control. Both the control and the experimental group consisted of 30 examinees of both sexes, age range 3–10 years. Statistically significant differences in AAP and GGT activities expressed as U/mmol creatinine were recorded after 12 days of cephalexin therapy in comparison with the control ($p < 0.01$). At the same time, no significant differences in NAG activity of the patients in relation to the control were observed during the entire course of the therapy. Based on the obtained results it can be concluded that treatment of 3–10 years old patients with the applied cephalexin doses for 15 days results in mild nephrotoxic changes close to the end of therapy accompanied by increased activities of AAP and GGT, the enzymes known as very sensitive indicators of nephrotoxicity. The results showing that during the entire period of cephalexin application no changes in NAG, as a lysosomal enzyme, were observed, could be taken as a proof that this antibiotic did not lead to severe injuries of epithelial proximal tubule cells at the level of cell organelles.

Keywords: alanine aminopeptidase (AAP), γ-glutamyltransferase (GGT), N-acetyl-β-D-glucosaminidase (NAG), urine, cephalexin

Kratak sadržaj: Radi određivanja nefrotoksičnosti cefalosporinskog antibiotika cefaleksina, praćena je aktivnost enzima dominantno lokalizovanih u ćelijama epitela proksimalnih tubula, alaninaminopeptidaze (AAP), gama-glutamil-transferaze (GGT) i N-acetil-beta-D-glukozaminidaze (NAG). Određivanje aktivnosti enzima je vršeno u uzorcima 12-časovnog urina kod 30 ispitanika kojima je, zbog grampozitivnih infekcija respiratornog i urinarnog trakta, *per os* apliciran cefaleksin u dozama od 50 mg/kg telesne mase dnevno za vreme sprovođenja terapije do 15 dana. Aktivnosti istih enzima su određivane i u 12-časovnom urinu 30 ispitanika kontrolne grupe. I eksperimentalna i kontrolna grupa sastojale su se od ispitanika oba pola, starosti od 3 do 10 godina. Statistički značajne razlike u aktivnostima AAP i GGT, izražene u U/mmol kreatinina, registrovane su između ispitanika eksperimentalne i ispitanika kontrolne grupe nakon dvanaestog dana sprovođenja terapije ($p < 0,01$). Aktivnost NAG ispitanika eksperimentalne grupe u odnosu na ispitanike kontrolne grupe se nisu značajno menjale za čitavo vreme petnaestodnevne terapije. Može da se zaključi da petnaestodnevni tretman ispitanika starosti od 3 do 10 godina preporučenim dozama cefaleksina izaziva blage nefrotoksične promene pri kraju terapije koje su praćene porastom aktivnosti AAP i GGT, veoma osetljivih indikatora nefrotoksičnosti. Za čitavo vreme sprovođenja terapije nije došlo do porasta aktivnosti lizozomalnog enzima NAG, što znači da ne dolazi do težih oštećenja ćelija epitela proksimalnih tubula na nivou organela.

Ključne reči: alaninaminopeptidaza (AAP), gama-glutamil-transferaza (GGT), N-acetil-beta-D-glukozaminidaza (NAG), urin, cefaleksin

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Introduction

Cephalexin represents a semisynthetic first generation cephalosporin antibiotic, applied in the therapy of moderate respiratory and urogenital infections. It has been shown to be efficient against numerous Gram-positive and some Gram-negative bacteria. Different from aminoglycoside antibiotics, its nephrotoxicity in humans as well as that of other cephalosporins was very seldom emphasized in scientific and professional publications. The nephrotoxicity of cephalexin was first examined in experimental animals (1, 2). However, investigations performed during the last decade clearly demonstrated that cephalexin can provoke in some patients acute tubular necrosis, primarily acute tubulointerstitial nephritis (3–5). Tubular necrosis results from changes in the cell membranes of proximal tubules leading to disturbances in organic ion transport across the cell membranes (6–8) due to the antibiotic binding to protein carriers of organic ions, acylation of target proteins involved in the transport and lipid peroxidation (9).

Increased enzymatic activities of proximal tubule epithelial cells AAP (EC 3.4.11.2), GGT (EC 2.3.2.2) and NAG (EC 3.2.1.52) in urine represent a very sensitive marker of acute renal impairment (10–12). However, publications related to cephalexin-induced enzymuria performed on a representative sample of examinees are lacking in the available literature. This prompted us to find out whether normally prescribed cephalexin doses lead to increased activities of the enzymes dominantly occuring in the epithelium of proximal tubule cells and whether some of these enzymes detected in urine of the patients could be used as an early indicator of cephalexin nephrotoxicity.

Material and Methods

Sixty children of both sexes, age range 3 to 10 years, were included in the present study. The examinees were patients at the Pediatric Hospital, Clinical Centre of Banjaluka (Banjaluka, Republic of Srpska, Bosnia and Herzegovina). Experimental group consisted of 30 examinees treated *per os* with daily doses of 50 mg cephalexin/kg body mass for 15 days against respiratory or urogenital infections. Control group included the same number of age-matched examinees with neither respiratory nor urogenital infections.

Morning, 12-h urine samples were collected and kept at –25 °C until the analyses. The data on age, sex and health condition of the examinees were introduced into a questionnaire when the first urine samples were taken.

Upon the separation of the enzymes contained in urine by gel filtration (13), enzymatic activities of AAP (14), GGT (15, 16) and NAG (17), as well as creatinine concentration (18) were determined by photometric methods and expressed as U/mmol creatinine.

The results, analysed by standard statistical methods, were expressed as means ± S.D. and graphically presented. Significance of the differences between the experimental and control group was determined by Student's *t*-test.

Results

Mean values of enzymatic AAP, GGT and NAG activities with standard deviations are depicted in *Figures 1–3*, respectively. As seen from *Figure 1*, a statistically significant increase of AAP activity in the urine samples of cephalexin-treated patients ($\bar{x}$ = 0.62 ± 0.23) in relation to the control group ($\bar{x}$ = 0.4 ± 0.15) was recorded on day 12 of the treatment ($p < 0.01$) and remained statistically significant to the end of the therapy.

The GGT activity in the urine samples of the experimental group ($\bar{x}$ = 7.18 ± 2.22) was also increased and the difference in relation to the control group ($\bar{x}$ = 3.73 ± 1.21) became statistically significant after 12 days of cephalexin application (*Figure 2*) and was stable till the end of the cephalexin therapy.

However, no significant changes in NAG activity were observed during the entire period of cephalexin application as compared to the control group (*Figure 3*).

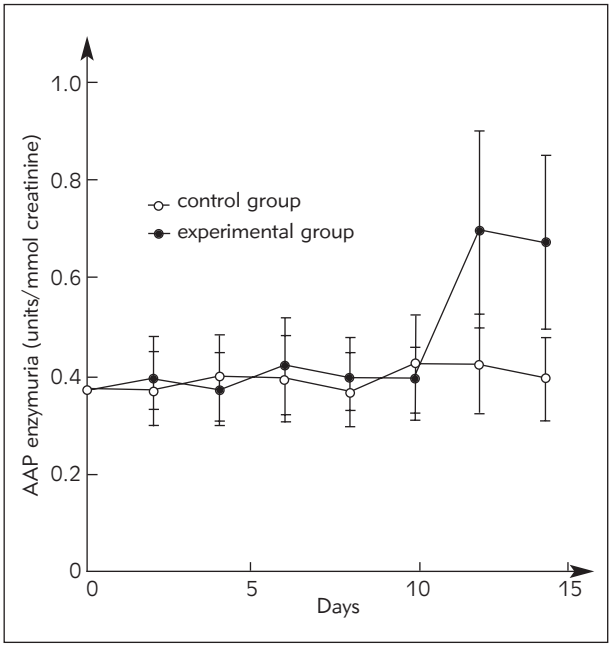

Figure 1 Kinetics of the changes in alanine aminopeptidase (AAP) activity in the urine of patients treated with cephalexin for 15 days and the corresponding control expressed as the means ± S.D.

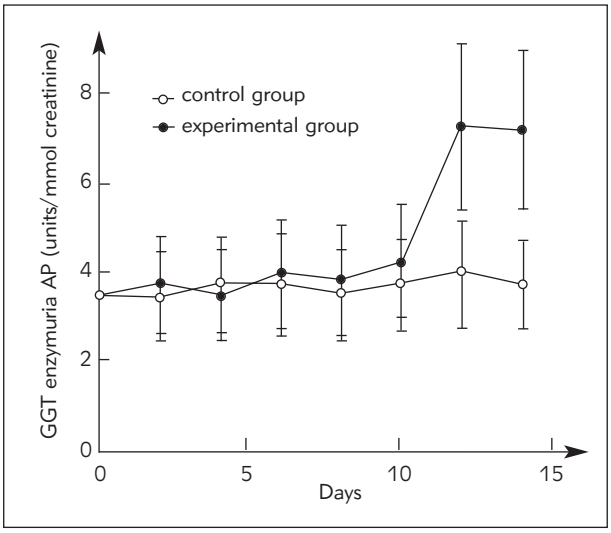

Figure 2 Kinetics of the changes in γ-glutamyltransferase (GGT) activity in urine of the patients receiving cephalexin for 15 days and the corresponding control expressed as the means ± S.D.

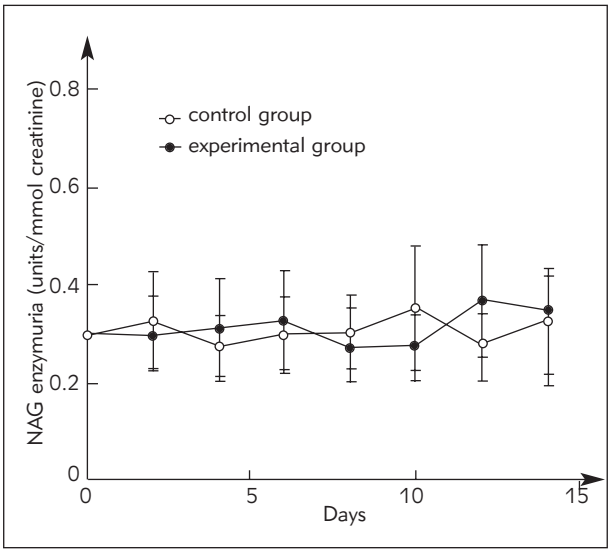

Figure 3 Kinetics of the changes in N-acetyl-β-D-glucosaminidase (NAG) activity in urine of the patients subjected to cephalexin therapy for 15 days and the corresponding control expressed as the means ± S.D.

Discussion

In the present study, cephalexin was orally applied for 15 days in daily doses of 50 mg/kg body mass to the patients (age range 3–10 years) suffering from respiratory or urogenital infections. In order to examine the possible nephrotoxic cephalexin action, the activities of two membrane enzymes of epithelial proximal tubule cells (AAP and GGT) as well as of a lysosomal enzyme (NAG) were determined. The results revealed a statistically significant cephalexin-induced increase in AAP and GGT activities on day 12 of the therapy (*Figures 1* and *2*). These findings clearly demonstrated that even the recommended cephalexin doses provoke acute injuries of proximal tubule cell membranes and pointed to the possibility of using some enzymes excreted in urine as reliable biomarkers of these injuries, what is in full accordance with the earlier reports of Trof et al. (19) and Lisowska-Myjak (11). The enzymes of proximal tubule cell membranes represent very sensitive indicators of nephrotoxicity, but our results revealed no changes at the level of proximal tubule cell membranes during the first 10 days of cephalexin therapy. We have found previously (20) that the changes observed on day 12 of the cephalexin therapy, seen as increased activity of both AAP and GGT and enzymuria, are at the beginning reversible.

No statistically significant changes in the activity of a lysosomal enzyme NAG during cephalexin application for 15 days in relation to the control were recorded (*Figure 3*). These results clearly demonstrated that cephalexin in the recommended doses caused no injuries at the level of proximal tubule organelles, which are as a rule irreversible, leading to apoptosis of proximal tubule cells (21, 22). Our results, revealing no significant changes in NAG activity in the urine samples of cephalexin-treated patients when compared to the corresponding control, differ from the data of Daghero et al. (23) who reported an increased NAG activity in 7% of the patients receiving some cephalosporin antibiotics. This discrepancy can be ascribed to the fact that Daghero et al. (24) examined enzymuria during therapy with several cephalosporins known to be more toxic than cephalexin.

On the basis of the results obtained throughout the present study, it can be concluded that normal, recommended cephalexin therapy for 15 days applied to 3–10-year-old patients with moderate, mainly Gram-positive infections of the respiratory or urinary tract, leads at the end of the therapy to statistically significant increase in the activity of AAP and GGT, the enzymes of epithelial proximal tubule cell membranes, known as very sensitive indicators of nephrotoxicity. At the same time, the activity of NAG, a lysosomal enzyme, remained at about the same level during the entire period of cephalexin application. This indicates that cephalexin therapy in the doses applied did not lead to severe injuries of the epithelial proximal tubule cells at the level of cell organelles. During the first 10 days of the therapy, no changes in the activity of the proximal tubule enzymes studied were observed and in the later stages of cephalexin application up to day 15 of the therapy, the changes appearing from day 12 on were reversible ones.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

References

1. Diaz OAJ, Sumano LH, Ocampo CL, Mateos TG. Evaluation of the nephrotoxicity of the administration of sodium cephalexin with gentamicin sulphate in dogs. Vet Mex 1995; 26: 247–9.
2. Plumb's Veterinary Drug Handbook, 5th ed., Blackwell Publishing. 2005; 206–9: 864–7.
3. Longstreth KL, Robbins SD, Chaing MD, Doe NS, Facp MD. Cephalexin-induced acute tubular necrosis. Pharmacotherapy 2004; 24: 808–11.
4. Rossert J. Drug-induced acute interstitial nephritis. Kidney Int 2001; 60: 804–17.
5. Alper AB. Nephritis, interstitial. Kidney Int 2006; 69: 213–17.
6. Shugarts S, Benet LZ. The role of transporters in the pharmacokinetics of orally administered drugs. Pharm Res 2009; 26: 2039–54.
7. Srimaroeng C, Perry JL, Pritchard JB. Physiology, structure and regulation of the cloned organic anion transporters. Xenobiotica 2008; 38: 889–935.
8. Grover A, Benet LZ. Effects of drug transporters on volume of distribution. AAPS 2009; 11: 250–61.
9. Tune BM. Nephrotoxicity of beta-lactam antibiotics: mechanisms and strategies for prevention. Pediatr Nephrol 1997; 11: 768–72.
10. Westhuyzen J, Endre ZH, Recce G, Reith DM, Saltissi D, Morgan TJ. Measurement of tubular enzymuria facilitates early detection of acute renal impairment in the intensive care unit. Nephrol Dial Transplant 2003; 18: 543–51.
11. Lisowska-Myjak B. Serum and urinary biomarkers of acute kidney injury. Blood Purif 2010; 29: 357–65.
12. Che M, Xue B, Dai H, Qian J, Ni Z, Axelsson J, et al. Clinical usefulness of novel biomarkers for the detection of acute kidney injury following elective cardiac surgery. Nephron Clin Prac 2010; 115: 66–72.
13. Werner M, Muruhn D, Atoba M. Use of gel filtration in the assay of urinary enzymes. J Chromatog 1969; 40: 254–63.
14. Jung K, Scholz D. An optimized assay of alanine aminopeptidase activity in urine. Clin Chem 1980; 26: 1251–4.
15. Persijn JP, Van der Slik W. A new method for determination of gamma-glutamyltransferase in serum. J Chem Clin Biochem 1976; 14: 421–7.
16. Szasz G. A kinetic colorimetric method for determination of gamma-glutamyltranspeptidase in serum. Z Klin Chem Klin Biochem 1974; 12: 228–32.
17. Maksime J, Saito E, Obuchi M, Kanayma M, Yosida U. Improved kinetic rate assay of urinary N-acetyl-ß-D-glucosaminidase with 2-chloro-4-nitrophenyl-N-acetyl-β-D-glucosaminidase as substrate. Clin Chem 1990; 36: 319–22.
18. Bartels H, Böhmer M. Eine Mikromethode zur Kreatininbestimmung. Clin Chim Acta 1971; 32: 81–5.
19. Trof RJ, Di Maggio F, Leemreis J, Goeneved AB. Biomarkers of acute renal injury and renal failure. Shock 2006; 26: 245–53.
20. Davidović-Plavšić B, Vujić T, Uletilović S, Predojević-Samardžić J, Malčić D, Saničanin Ž. Urinary activities of proximal tubule enzymes in neonates treated with gentamicin. Journal of Medical Biochemistry 2010; 29: 44–7.
21. Davidović B, Predojević-Samardžić J, Uletilović S, Malčić D, Saničanin Ž. Activities of proximal tubule enzymes in urine of patients treated with gentamicin. J Med Biochem 2007; 26: 46–50.
22. Ćorić V, Plješa-Ercegovac M, Matić M, et al. The role of GSTM1 and GSTT1 polymorphism in patients with ranal cell carcinoma. Journal of Medical Biochemistry 2010; 29: 204–10.
23. Daghero O, Andreoni G, Arione R, Bendiscioli L, Bramato C, Cimino T, et al. Cephalosporins and enzymuria. Minerva Med 1986; 77: 231–7.
24. Cunha BA. Third generation of cephalosporins: a review. Clin Therap 1992; 14: 616–52.

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