Original Article

Effect of Vitamin E on Serum Urea Level on Gentamicin Induced Nephrotoxicity in Long Evans Rats

Md. Shameem Ahmed[1], Mohammad Ashraf Ahmed[2], Md. Rezwanur Rahman[3], Ashrafuzzaman[4], Mushtari Jahan[5], Md. Abdul Matin[6], Aysha Yasmin[7], Marjina Khatun[8], Farhana Afroz[9], Kuazi Dil Afroz[10]

Abstract

Background: *The kidneys have an important role in eliminating the final products of metabolic activities, excreting the drugs and chemicals. A variety of frequently used drugs have been demonstrated to produce nephrotoxic effects.* ***Objective:*** *This study was carried out to observe the effect of vitamin E on gentamicin-induced nephrotoxicity by assessing serum urea level in Long Evans rats.* ***Materials and method:*** *The experimental study was carried out on 40 healthy Long Evans rats of both sex with the weight ranges from 172-255 gm and the age ranges from 7 to 10 weeks. The rats were divided into four groups - Group A (normal control) received normal saline, group B, C and D received gentamicin for 6 days, rats of group C received vitamin E capsule for total 9 days with gentamicin whereas group D received vitamin E capsule for total 10 days with gentamicin. Serum urea level was measured at the end of the experiment.* ***Results:*** *The (mean±SD) serum urea levels in group A, B, C and D were 4.79±0.32, 12.41±1.22, 7.56±1.11 and 7.15±1.09 mmol/L respectively. The differences between groups were highly significant ($p<0.001$) for group A & B, A & C, A & D, B & C, B & D whereas the difference between C & D ($p>0.01$) was not significant. Serum urea level of the normal saline control group (group A) was within the normal limit (4.79 mmol/L). Serum urea level in gentamicin treated rats (group B) was more in comparison to gentamicin and vitamin E treated rats (group C & D) and pretreatment with longer duration group (group D) showed lower serum urea value than shorter one (group C) though the groups showed no significant difference.* ***Conclusion:*** *Vitamin E treatment showed some protective effect against gentamicin-induced nephrotoxicity. The results also indicated that effectiveness of vitamin E depends on duration of pretreatment that means the pretreatment duration must be increased to a suitable period for better protection against gentamicin-induced nephrotoxicity.*

Keywords: *Gentamicin; vitamin E nephrotoxicity.*

Delta Med Col J. Jan 2019;7(1): 11 – 15

1. Professor and Head, Dept. of Anatomy, Kumudini Women's Medical College, Mirzapur,Tangail, Bangladesh.
2. Assistant Professor, Dept. of Pharmacology, Kumudini Women's Medical College, Mirzapur,Tangail, Bangladesh.
3. Professor, Dept. of Biochemistry, Delta Medical College, Dhaka, Bangladesh.
4. Professor and Head, Dept. of Anatomy, Chittagong Medical College, Chittagong, Bangladesh.
5. Dental Surgeon, Dhaka, Bangladesh.
6. Associate Professor, Dept. of Paediatrics, Kumudini Women's Medical College, Mirzapur,Tangail, Bangladesh.
7. Associate Professor and Head, Dept. of Physiology, KhwajaYunus Ali Medical College, Enayetpur, Sirajgonj, Bangladesh.
8. Assistant Professor, Dept. of Community Medicine, Kumudini Women's Medical College, Mirzapur,Tangail, Bangladesh.
9. Assistant Professor, Dept. of Paediatrics, Kumudini Women's Medical College, Mirzapur,Tangail, Bangladesh.
10. Associate Professor, Dept. of Pathology, Kumudini Women's Medical College, Mirzapur,Tangail, Bangladesh.

Correspondence: Dr. Md. Shameem Ahmed. e-mail: drshameem007@gmail.com

Introduction

The kidneys are one of the vital organs of our body. Kidney is an important organ because of its role in eliminating the final products of metabolic activities, excreting the drugs and chemicals.[1,2] A variety of drugs have been demonstrated to produce nephrotoxic side effects. With regard to intrarenal structural processes, several factors make the kidney especially susceptible to toxic injury. The high rates of delivery of compounds to the kidney, concentration of drugs in tubule lumens and interstitial, and transcellular transport of toxins by the kidney make the renal tubular cells especially vulnerable to toxic injury and causes toxic nephropathy.[3] Acute renal failure complicates approximately 5% of hospital admissions and up to 30% of admissions to intensive care units. Gentamicin causes acute renal failure in 10% to 15% of all cases. The incidence of renal involvement due to gentamicin is estimated about 4 to 10%. Some of the patients treated for the syndrome of acute renal failure had received gentamicin before the onset of the syndrome.[4-7]

Gentamicin is the aminoglycoside of first choice because of its low cost and its reliable activity against all but the most resistant gram negative aerobes. This drug is excreted mainly in urine.[6] The renal clearance of gentamicin averages 85% of total clearance, indicating that the drugs are primarily eliminated by kidney.[8] The range between effective and toxic blood level is narrow. Nephrotoxic damage in aminoglycoside-treated patients has increased from 5 to almost 40% and patient may experience renal damage even in the use of recommended doses and therapeutic serum concentrations.[9-10] It is apparent that gentamicin nephrotoxicity is reversible but that renal function may take up to several months to return to pre-treatment values.[11]

Vitamin E is an antioxidant and probably acts as a free radical scavenger in cell membranes to protect membrane polyunsaturated fatty acids from peroxidation.[12] Vitamin E is used in this study because dietary vitamin E supplementation ameliorates renal injury in chronic puromycin aminonucleoside nephropathy, and suppresses peroxidation of membrane phospholipids.[13,14] The important cause of cell damage is injury induced by accumulation of such free radicals in the body.[15] Very recently it was found that vitamin E protects against gentamicin-induced nephrotoxicity in rats.[16,17]

Materials and method

This experimental study was carried out on 40 healthy Long Evans rats of both sex with the weight ranges from 172-255 gm and the age ranges from 7 to 10 weeks. They were allowed to live at normal room temperature under the condition of normal natural light and dark schedule and were fed on pellets of standard rat food. Drugs used were gentamicin, vitamin E and normal saline (0.9%). Distilled water was used as vehicle for both the drugs.

The rats were divided into four groups and randomly selected. Grouping of the rats was done according to treatment pattern.

Group A (Normal control)

The rats of this group received normal diet and injection normal saline intramuscularly (2 mL/kg/day) for 6 days and no drug treatment were given.

All the rats were sacrificed on the 7th day.

Group B (Experimental control)

The rats of this group received normal diet and injection gentamicin intramuscularly (80 mg/kg/day) for 6 days.

All the rats were sacrificed on the 7th day.

Group C (Experimental)

The rats of this group received normal diet and

vitamin E capsule orally (1mg/g bw/day) for total 9 days (pretreatment for 3 days + along with gentamicin for 6 days) and gentamicin were injected intramuscularly (80 mg/kg/day) during the last 6 days of the 9-day period.

All the rats were sacrificed on the 10th day that is 24 hours after gentamicin administration.

Group D (Experimental)

The rats of this group received normal diet and vitamin E capsule orally (1mg/g bw/day) for total 10 days (pretreatment for 4 days + along with gentamicin for 6 days) and gentamicin were injected intramuscularly during the last 6 days of the 10-day period.

All the rats were sacrificed on the 11th day that is 24 hours after gentamicin administration.

The animals were sacrificed on the fixed day of each group under chloroform anaesthesia by cervical dislocation. Kidneys were collected after opening the abdomen. Blood was obtained by cardiac puncture by 5 cc syringe from each rat in separate test tubes and allowed to clot for 1 hour, at room temperature. The serum was separated and preserved. Estimation of serum urea was done by chemical method that is Diacetylmonoxime method. Data were analyzed by SPSS version 12.0 for Windows. This study was conducted in the department of Anatomy in Dhaka Medical College, Dhaka, Bangladesh.

Results

The (mean±SD) serum urea levels in group A, B, C and D were 4.79±0.32, 12.41±1.22, 7.56±1.11 and 7.15±1.09 mmol/L respectively. The differences between groups were highly significant ($p<0.001$) for group A & B, A & C, A & D, B & C, B & D, whereas the difference between C & D ($p>0.01$) was not significant.

The result indicates that serum urea level was more in gentamicin treated rats (group B) in comparison to simultaneous vitamin E (group C & D) treated rats but the urea level is high in relation to normal saline control group (group A). In the present study the results indicate that serum urea level of normal saline control group (group A) of rats was within normal limit (4.79 mmol/L). But serum urea level was abnormally high (12.41 mmol/L) in the gentamicin treated rats (group B). On the other hand simultaneous vitamin E treated rats showed that the values are relatively lower in comparison to only gentamicin treated rats. But the values of serum urea level in group C and D were significantly higher ($p<0.001$) in comparison to normal saline control rats (group A).

Table I: Distribution and comparison of serum urea levels in different group of rats

Group (n=10 in each)		Serum urea in mmol/L (Mean±SD)	
A		4.79±0.32(4.30-5.40)	
B		12.41 ± 1.22(10.80-14.60)	
C		7.56±1.11(5.30-8.70)	
D		7.15±1.09(5.30-8.60)	
p values			
A vs B	<0.001*	B vs C	<0.001*
A vs C	<0.001*	B vs D	<0.001*
A vs D	<0.001*	C vs D	>0.10^{ns}

Values in parenthesis indicates range

Statistical analysis done by ANOVA (multiple comparisons)

ns = not significant

*= significant

Group A : Normal Control (normal saline only)

Group B : Experimental control (gentamicin only)

Group C : Experimental (vitamin E 9 days + gentamicin for last 6 days)

Group D : Experimental (vitamin E 10 days + gentamicinfor last 6 days)

Discussion

In spite of being one of the most nephrotoxic aminoglycoside, gentamicin is still frequently used as a first and second choice drug in a vast variety of clinical situations. Moreover, this aminoglycoside has been widely used as a model to study the nephrotoxicity of this family of drugs, both in experimental animals and human being.[18]

However, due to its toxicities, prolonged use is restricted to the therapy of life threatening infections.[19]

The present study demonstrated the severity of nephrotoxicity by assessing serum urea level in different group of rats. The mean serum urea level was significantly raised in all the groups treated with gentamicin in comparison to normal control group. Alarifi et al.[20] reported that serum urea was significantly increased in the gentamicin treated groups as compared to normal saline control group. This was the biochemical proof of nephrotoxicity produced by gentamicin and conforms to the findings of Cohen et al.[21], Erdem et al.[22], Sepehri et al.[23], Babu et al.[24], Kore et al.[25] and many other researchers.

Abdel-Naim et al.[16] found that pretreatment with intramuscular vitamin E at a dose of 250 mg/kg/day for 3 consecutive days prior to gentamicin administration significantly lowered the elevated serum urea and creatinine levels. In the current study also vitamin E treatment showed some protective effect against gentamicin-induced nephrotoxicity induced by gentamicin administered during the last 6 days of treatment with vitamin E by returning the urea concentration to near normal. The results also indicate that vitamin E was less effective in less duration of pretreatment that means the pretreatment duration must be increased to a suitable period for better protection against gentamicin induced nephrotoxicity.

References

1. Dyson M. Urinary system. In: Bannister LH, Berry MM, Collins P, Dyson M, Dussek JE, Ferguson MWJ, editors. Gray's Anatomy: The Anatomical Basis of Medicine and Surgery. 39th ed. London: Churchill Livingstone; 2005. p.1813-46.
2. Cronin RE, Henrich WL. Toxic Nephropathy. In: Brenner BM, editor. The kidney. 5th ed. London: WB Saunders Company; 1996. p.1680-84.
3. Humes HD, Nguyen VD. Acute Renal Failure and Toxic Nephropathy. In: Klahr S, Massry SG, editors. Contemporary Nephrology. Springer: Boston, MA; 1987. p.401-402.
4. Brady HR, Brenner BM, Lieberthal W. Acute Renal Failure. In: Brenner BM, editor. The Kidney. 5th ed. U.S.A: WB Saunders Company; 1996. p.1200-10.
5. Humes HD, Sastrasinh M, Weinberg JM. Calcium is a Comparative Inhibitor of Gentamicin-Renal Membrane Binding Interactions and Dietary Calcium Supplementation Protects against Gentamicin Nephrotoxicity. J Clin Invest. 1986;73:134-47.
6. Falco FG, Smith HM, Arcieri GN. Nephrotoxicity of Aminoglycosides and Gentamicin. J Infect Dis. 1969;119:406-409.
7. Schultze RG, Winters RE, Kauffman H. Possible Nephrotoxicity of Gentamicin. J Infect Dis. 1971;124 Suppl:S145-47.
8. Simon VK, Mosinger EU, Malerczy V. Pharmacokinetic Studies of Tobramycin and Gentamicin. Antimicrob Agents Chemother. 1973:445-50.
9. Dahlgren JG, Anderson ET, Hewitt WL. Gentamicin Blood Levels: A Guide to Nephrotoxicity. Antimicrob Agent Chemother. 1975;58-62.
10. Schentag JJ, Plaut ME, Cerra FB. Comparative Nephrotoxicity of Gentamicin and Tobramycin: Pharmacokinetic and Clinical Studies in 201 Patients. Antimicrob Agents Chemother. 1981:859-66.
11. Luft FC. Clinical Significance of Renal Changes Engendered by Aminoglicosides in Man. J Antimicrob Chemother. 1984;13 Suppl A:S23-30.
12. Baigent MJ. Vitamin E. In: Harold K, Roschlau WHE, editors. Principles of Medical Pharmacology. 6th ed. New York: Oxford University Press; 1998. p.839.
13. Trachtman H, Schwob N, Maesaka J, Valderrama E. Dietary Vitamin E Supplementation Ameliorates Renal Injury in Chronic Puromycin Aminonucleoside Nephropathy. J Am Soc Neprol. 1995;5(10):1811-19.
14. Leung HW, Vang MJ, Mavis RD. The Cooperative Interaction between Vitamin E and Vitamin C in Suppression of Peroxidation of Membrane Phospholipids. Bio Biophy Acta. 1981;664:266-72.

15. Choudhury SAR. Vitamin E the Scavenger of Free Radicals in Health and Diseases: an Editorial. Bangladesh J Physiol Pharmacol 1996;12(1):1-2.

16. Abdel-Naim AB, Abdel-Wahab MH, Foad AF. Protective Effects of Vitamin E and Probucol against Gentamicin Induced Nephrotoxicity in Rats. Pharmacol Res. 1999;40(2):183-87.

17. Kadkhodaee M, Khastar H, Faghihi M, Ghaznavi R, Zahmatkesh M. Effects of Co Supplementation of Vitamins E and C on Gentamicin-Induced Nephrotoxicity in Rat. Exp Physiol. 2005;90(4):571-76.

18. Lopez-Novoa JM, Quiros Y, Vicente L, Ana I. Morales AI, Lopez-Hernandez FJ. New Insights into the Mechanism of Aminoglycoside Nephrotoxicity: An Integrative Point of View. Kidney International. 2011;79(1):33-45.

19. Xie J, Talaska AE, Schacht J. New Developments in Aminoglycoside Therapy and Ototoxicity. Hear Res. 2011;281(1-2):28-37.

20. Alarifi S, Al-Doaiss A,Alkahtani S, Al-Farraj SA, Al-Eissa MS, Al-Dahmash B, et al. Blood Chemical Changes and Renal Histological Alterations Induced by Gentamicin in Rats. Saudi Journal of Biological Sciences. 2012;19(1):103-10.

21. Cohen L, Lapkin R, Kaloyanides GJ. Effect of Gentamicin on Renal Function in the Rat. J Pharmacol Exp Ther. 1975;193(1):264-73.

22. Erdem A, Gündogan NÜ, Usubütün A, Kilinç K, Erdem R, Kara A, et al. The Protective Effect of Taurine against Gentamicin-Induced Acute Tubular Necrosis in Rats. Nephrol Dial Transplant. 2000;15:1175-82.

23. Sepehri G, Derakhshanfar A, Saburi L. Does Propylthiouracil Increase the Gentamicin-Induced Nephrotoxicity in Rat? Iran J Basic Med Sci. 2013;16(11):1190-95.

24. Babu SV, Urolagin DK, Veeresh B, Attanshetty N. Anogeissuslatifolia Prevents Gentamicin Induced Nephrotoxicity in Rats. Int J Pharm. Sci. 2011;3(1):1091-95.

25. Kore KJ, Shete RV, Kale BN, Borade AS. Protective Role of Hydroalcoholic Extract of Ficus Carica in Gentamicin Induced Nephrotoxicity in Rats. Int J Pharm Life Sci. 2011;2:978-82.