Original Article

Effect of Vitamin E on Serum Creatinine level on Gentamicin Induced Nephrotoxicity in Long Evans rats

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Abstract

Background: *The kidneys are one of the vital organs of our body to excrete metabolic waste products, drugs and chemicals in the form of urine.*

Objectives: *This study was carried out to observe the effect of Vitamin E on gentamicin-induced nephrotoxicity by assessing serum creatinine level in Long Evans rats.*

Materials and Methods: *The experimental study was carried out on 40 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 (control) received normal saline, group B, C and D received gentamicin for 6 days, rats of group C received vitamin E capsule for 9 days with gentamicin whereas, group D received vitamin E capsule with gentamicin for total 10 days. Serum creatinine level was measured at the end of experiment.*

Results: *The mean (mean+ SD) serum creatinine levels in group A, B, C and D were 0.98+0.34, 2.36+ 0.44, 1.39+0.18 and 1.30+0.18 respectively. The differences between the groups were highly significant ($p<0.001$) in group A&B, B&C and B&D whereas the difference between C&D was not significant ($p>0.50$). Serum creatinine level on the normal saline control (group A) was within normal limit (0.98mg/dl). Serum creatinine level in gentamicin treated (group B) rats were more in comparison to gentamicin and vitamin E treated rats (group C&D) and pretreatment with longer duration group (group D) showed lower serum creatinine level than the shorter one (group C) though there was no significant difference.*

Conclusion: *Vitamin E treatment showed some protective effects against gentamicin induced nephrotoxicity. The results also indicated that effectiveness of vitamin E depends on a suitable duration of pretreatment for better protection against gentamicin-induced nephrotoxicity.*

Key words: *Vitamin E, Gentamicin induced nephrotoxicity, serum creatinine level.*

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Introduction

The kidneys are known as the excretory organ of human body. Like the liver and lungs, the kidneys retrieve essential materials and dispose the wastes.[1,2] The final urine contains water and electrolytes such as urea, uric acid, creatinine and breakdown products of various substances, drugs and chemicals.[3,4] Kidney also regulates volumes and composition of body fluids.[5] A variety of drugs have been demonstrated to produce nephrotoxic side effects.[6,7] Drug induced nephrotoxicity can lead to acute renal failure.[8] Acute renal failure is an important cause of morbidity and mortality in Bangladesh.[9] Gentamicin causes acute renal failure in 10% to 15% of all cases.[10] The incidence of renal involvement due to gentamicin, was estimated about 4% to 10% in patients treated for the syndrome of acute renal failure who had received gentamicin before the onset of the syndrome.[11,12]

Gentamicin is the first choice aminoglycosides because of its low cost and reliable activities against gram negative aerobes.[13] This drug is excreted mainly through urine.[14] The renal clearance of gentamicin averages 85% of total clearance, indicating that the drug is primarily eliminated by the kidneys.[15] The range between effective and toxic blood level is narrow thus, the nephrotoxic effects in aminoglycoside treated patients has increased more than 40% even it is used as recommended doses and in therapeutic concentrations.[16] It is apparent that, gentamicin nephrotoxicity is reversible but the renal function may take up to several months to return to pre-treatment values.[17] Vitamin E is an anti-oxidant and act as free radical scavenger in cell membranes to protect membrane poly unsaturated fatty acids from peroxidation.[18]

Vitamin E is used in this study, because dietary vitamin E supplementation ameliorates decreases renal injury in chronic

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puromycin aminonucleoside nephropathy and suppresses the peroxidation of cell membrane phospholipids.[19,20] Very recently it was found that vitamin E protects against gentamicin induced nephrotoxicity in rats.[21]

Materials and Methods

This experimental study was carried out on 40 healthy long Evans rats of both sex with the weight ranges from 172-255 grams and the age ranges from 7 to 10 weeks. They were allowed to live on normal room temperature under the conditions of normal natural light and dark schedule and were fed on pellets of standard rat foods. Drugs used were gentamicin, vitamin E and normal saline (0.9% NaCl)). Distilled water was used as vehicle for both the drugs. The study was carried out in the Department of Anatomy, Dhaka Medical College (DMC) Dhaka, during the period from February 2005 to January 2006. The rats were divided into four groups and randomly selected. Grouping of the rats were done according to treatment pattern.

Group A (control)
The rats of this group received normal diet and injections of normal saline intramuscularly (2ml/kg/day) for 6 days without any drugs treatment. All the rats were sacrificed on 7th day.

Group B (Experimental control)
The rats of this group received normal diet and injection of gentamicin intramuscularly (80mg/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 03 days + along with gentamicin for 6 days) and gentamicin was injected intramuscularly (80mg/kg/day) during the last 06 days of 9-day period. All the rats were sacrificed on 10th day that is 24 hours after administration of gentamicin.

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 04 days + along with gentamicin for 6 days) and gentamicin was injected intramuscularly (80mg/kg/day) during the last 06 days of the 10-day period. All the rats were sacrificed on 11th day that is 24 hours after administration of gentamicin.

The animals were sacrificed on the fixed day under chloroform anesthesia by cervical dislocation. Kidneys were collected after the opening of abdomen. Blood was obtained by cardiac puncture by 5cc syringe from each rat in separate test tubes and allowed to clot for one 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. Standard deviation (SD) and mean of the collected Data were calculated for paired student's 't' test and comparison between the groups were made by using ANOVA test. The study was conducted in the Department of Anatomy in Dhaka Medical College, Dhaka, Bangladesh.

Results

The mean (mean+ SD) serum creatinine levels in group A, B, C and D were 0.98+0.34, 2.36+ 0.44, 1.39+0.18 and 1.30+0.18 respectively. The differences between the groups were highly significant ($p<0.001$) in group A&B, B&C and B&D. but difference between C&D was not significant ($p>0.50$). In Table-II indicated that serum creatinine level was more in gentamicin treated rats (Group B) in comparison to simultaneous vitamin E treated rats (group C&D) but the creatinine level was high in relation to normal saline control group (group A). Present study In Table-II also showed that serum creatinine level on normal saline control group (group A) was within normal limit (0.98±0.34). But serum creatinine level was abnormally high (2.36mg/dl) in gentamicin treated rats (group B). In Table-I On the other hand, simultaneous vitamin E treated rats showed, that the values are relatively lower in comparison to only gentamicin treated rats (group B). But the values of serum creatinine level in group C and D were significantly higher ($p<0.001$) in compare to normal saline group rats (group A).

Table I: Grouping of rats, dose of drugs and vehicle, duration of treatment and sacrifice schedule.

Groups	Number of rats (n)	Drugs	Dose	Route of administration	Pre treatment Vit E	Simultaneous treatment	Total duration	Time of sacrifice (24 hours after the last dose)
Group A (Normal Control)	10	0.9% Normal Saline	2 ml/kg/day	I M	-	Day 5 - day 10	6 days	7th Day
Group B (Experimental control)	10	Gentamicin	80 mg/kg/day	I M	-	Day 5 - day 10	6 days	7th Day
Group C (Experimental)	10	Vitamin E and Gentamicin	1 mg/gm body weight	Oral	Day 1 - day3	Day4 - day9	9 days	10th Day
			80 mg/kg/day	IM		Day4 - day9	6 days	
Group D (Experimental)	10	Vitamin E and Gentamicin	1 mg/gm body weight	Oral	Day1 - day4	Day5 - day10	10 days	11th Day
			80 mg/kg/day	IM		Day5 - day10	6 days	

I M: Intramuscular

Table II: Serum creatinine levels in different group of rats

Group (n=10)	Serum creatinine in mg/dl (Mean±SD)
A	0.98±0.34 (0.60-1.60)
B	2.36±0.44 (1.70-3.10)
C	1.39±0.18 (1.10-1.70)
D	1.30±0.18 (1.10-1.60)

P value					
A vs B	<0.001***	B vs C	<0.001***	C vs D	>0.50^{ns}
A vs C	<0.01**	B vs D	<0.001***		
A vs D	<0.05*				

Values in parenthesis indicates range

Statistical analysis done by ANOVA (multiple comparisons)
ns = not significant
*/**/*** = significant
Group A : Control (normal saline only)
Group B : Experimental control (gentamicin only)
Group C : Experimental (vitamin E + gentamicin)
Group D : Experimental (vitamin E + gentamicin)

Discussion

Gentamicin is still used as a first and second choice of drug in vast varieties of clinical practice despite of having nephrotoxic effects. Moreover, this aminoglycoside has been widely used as a model to study nephrotoxicity of this family of drugs, both in experimental animals and in human beings.[22]

Present study revealed the severity of nephrotoxicity by assessing the serum creatinine level among different group of rats. It was found that, the mean serum creatinine level was high in gentamicin treated rats than comparison to normal control group. Similar reports were also observed in several studies.[23,24] This biochemical proof of nephrotoxicity produced by gentamicin was again found in number of research works.[25,26]

In present study, it was observed that vitamin E treated rats showed lower level of serum creatinine than that of only gentamicin induced nephrotoxicity rats. Abdel-Naim et al.[27] reported the similar findings that intramuscular administration of vitamin E at a dose of 250mg/kg/day once daily for 03 consecutive days prior to gentamicin administration had significantly lowered the serum creatinine level. Similar finding was again seen in some studies.[28,29]

Conclusion

Current study showed that vitamin E has partial protection against gentamicin induced nephrotoxicity by returning the serum creatinine level to normal. The results also indicated that, vitamin E was less effective in lesser duration of pretreatment. So, it was proved that for better protection against gentamicin induced nephrotoxicity, pretreatment with vitamin E must have an increased suitable duration. However, further study with large sample and different doses of vitamin E for different period of time is recommended.

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