TO EVUALATE THE AMELIORATIVE ROLE OF VITAMIN E ON NEPHROTOXICITY INDUCED BY VANCOMYCIN IN RABBITS

Shabana Ali, Mahwish Qaisrani*, Kulsoom Farhat, Akbar Waheed, Muhammad Faisal Afridi***, Muhammad Wasiullah Khan**

Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Foundation University of Medical College, Islamabad Pakistan, **Islamic International Medical College, Rawalpindi Pakistan, ***CMH Multan Institute of Medical Sciences, Multan/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To analyze the ameliorative effects of vitamin E on vancomycin-induced nephrotoxicity in rabbits.
Study Design: Laboratory based experimental study.
Place and Duration of Study: National Institute of Health, Islamabad and Department of Pharmacology and Therapeutics, Army Medical College, Rawalpindi, from Jul to Aug 2017.
Methodology: Study included seventy adult, male and female, rabbits weighing 1-2 kg. Group A served as a control group (n=10), group B (n=30) received vancomycin, 200mg/kg I/*p* dissolved in normal saline twice a day for seven days while group C (n=30) received oral vitamin E, 500mg/kg 5 days prior to vancomycin administration and for seven days after 200mg/kgI/*p* vancomycin administration. On the eighth day animals were sacrificed. Blood samples were drawn on day 0 and day 8. Estimation of kidney damage was assessed via serum urea, creatinine, sodium and potassium levels. Kidneys were assessed for histopathological examination.
Results: Vitamin E administration was buoyant in alleviating marked nephrotoxicity imposed by vancomycin (group B) by significantly decreasing serum urea ($p<0.001$), creatinine, ($p<0.001$), serum sodium ($p<0.001$) and serum potassium levels ($p<0.001$). Improvement in the renal morphology was also witnessed in group C.
Conclusion: Vitamin E successfully ameliorated the nephrotoxicity induced by vancomycin administration. This implicates the therapeutic importance of vitamin E as a nephroprotective agent.

Keywords: Antioxidants,Vitamin E, Vancomycin Induced, Nephrotoxicity.

INTRODUCTION

Methicillin resistant *Staphylococcus Aureus* (MRSA) and Enterococci are some of the causative agents which lead to serious and difficult to treat infections[1]. There are a number of antimicrobial agents available for the treatment of these infections and among them vancomycin proves to be the most effective and the drug of choice for resistant cases[2]. Although, it has high antimicrobial activity, low risk, synergism with beta lactam antibiotics, there were reports of treatment failure with vancomycin due to suboptimal therapy[3]. To avoid poor outcomes, it has been recommended to use high doses of vancomycin and to maintain trough levels between 10-20 ug/ml. Several studies have established relationship between vancomycin trough levels and the occurrence of nephrotoxicity. However, the nephrotoxic potential with such high dose can limit its use[4]. The precise mechanism involved in vancomycin induced nephrotoxicity has not been recognized. The major site for vancomycin reabsorption is proximal renal tubules[5], therefore, proximal renal tubular ischemia is more evident. Several mechanisms have been proposed for nephrotoxicity including lipid peroxidation, inflammatory processes, enhances oxygen consumption due to increased ATP levels of renal cellsand production of oxygen free radicals imposing oxidative stress and hence, renal damage[6]. This substantiation led to quantify the nephroprotective role of antioxidants.

One such propitious endogenous lipophilic antioxidant agent is Vitamin E (alpha tocopherol)[7]. The biological activity of vitamin E is due to the presence of alpha tocopherol which

Correspondence: Dr Shabana Ali, Department of Pharmacology, Army Medical College Rawalpindi Pakistan
Email: shabana70_ali@yahoo.com
Received: 01 Aug 2018; revised received: 26 Jun 2019; accepted: 12 Jul 2019

hasradical scavenging action by inhibiting lipid peroxidation and provides protection from negative effects on renal cells[8]. Its role in ameliorating Gentimycin[9], Amikacin[10] Colistin and cyclophosphamide induced nephrotoxicity has been established. We designed this study to verify the nephroprotective role of vitamin E in vancomycin induced nephrotoxicity in rabbits.

The aim of the study was to elucidate the protective role of vitamin E in ameliorating vancomycin induced nephrotoxicity. The data derived would validate the future implication of vitamin E in clinical setup and treatment modalities.

METHODOLOGY

This study was conducted at the Department of Pharmacology & Therapeutics, Army Medical College Rawalpindi in collaboration with Pathology Department, Army Medical College and National Institute of Health, Islamabad, from July to August 2017. All procedures were approved by the Ethical Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi. Vancomycin was purchased from Abbott Laboratories, Karachi, Pakistan and Vitamin E from Martin Dow, Karachi, Pakistan.

This laboratory based experimental study included adult rabbits, both male and female, weighing approximately 1 to 2 kg for experimental study were obtained from National Institute of Health (NIH), Islamabad. They were allowed to acclimatize to the new environment for a week at animal house of NIH under standard laboratory conditions (12 hour light/dark cycle, 24 C and 50-70% humidity). Commercial standard food (carrots, choker and grains) and tap water was provided ad libitum. A total of seventy animals were selected and randomly divided into three groups as follows: (1) group A (n=10) served as control, received single intraperitoneal injection of 1 ml normal saline for seven days, (2) group B (n=30), toxic groupwas given vancomycin 200mg /kg intraperitoneally[11-13] twice a day for seven days, (3) group C (n=30) was given vitamin E 500 mg/kg orally[14] five days prior to vancomycin and every day at the same time for the next seven days. The animals were weighed on daily basis to adjust the dose. The duration of study was eight days for all the groups.

Blood samples were collected from marginal vein on day zero and on the eighthday by non-probability convenience sampling technique. They were sent for biochemical analysis for the determination of serum creatinine and urea by colorimetic Jaffe's and urease and glutamate dehydrogenase principle respectively. While serum sodium and potassium were analyzed on the principle of ion selective electrodes using Easylyte. On the eighth day animals were euthanized by the administration of ketamine hydrochloride 50mg/kg intraperitoneally Kidneys were dissected out immediately and washed with tap water to remove the excess blood. They were placed in 10% formalin solution after being split sagitally and embedded in paraffin for next 24 hours for fixation. The kidney tissues, about 5 micro meter thick were fixed on glass slides and after staining with haematoxylin and eosin, histopathological evaluation was done under light microscope according to the following criteria[15]. 0=indistinguishable from controls, 1=minimal, ≤25% tubules affected, 2 = mild, >25% ≤50% tubules affected, 3 = moderate, > 50% ≤ 75% tubules affected, 4 = marked, > 75% tubules affected.

The data were presented as Means ± SD. Statistical analysis of all the results was done using Microsoft Office Excel 2009 and SPSS version 21. To analyze the data of biochemical variables on day 0 and day eight, paired sample t-test was applied. *p*-value of <0.05 was considered statistically significant.

RESULTS

Seven days of treatment with vancomycin, group B, (200 mg/kg/body wt) produced remarkable nephrotoxicity as compared to the group A, characterized by an increase in serum urea, serum creatinine and derangements in serum sodium and serum potassium levels. While serum analysis showed significant decrease in all

the parameters in group C (table-I). Normal renal parenchymal morphology was seen in all of animals in the control group A (fig-1) (A). The histopathological findings revealed marked (90%) (table-II) renal tissue damage with tubular necrosis, atrophy and interstitial inflammation in most of the slides of group B which received 200mg/kg vancomycin (fig-1) (B). These prominent morphological effects were ameliorated by vitamin E and renal histopathological assessment exhibited negligible inflammatory changes with 23% and 63% animals showing minimal and mild changes respectively and only 6% exhibiting marked and moderate changes (fig-1C).

Table-I: The comparison of biochemical parameters between Group A, Group B and Group C.

Parameters	Group A			Group B			Group C		
	Day 0	Day 8	*p*-value	Day 0	Day 8	*p*-value	Day 0	Day 8	*p*-value
Serum Urea (mmol/L) (Mean ± SD)	4.7 ± 0.61	4.5 ± 0.49	0.177**	4.6 ± 0.70	7.9 ± 1.30	0.001*	5.3 ± 0.55	5.2 ± 0.55	0.001*
Serum Creatinine (µmol/L) (Mean ± SD)	80.5 ± 6.13	82.4 ± 7.41	0.127**	80.8 ± 7.7	94.5 ± 6.83	0.001*	83.96 ± 6.06	81.33 ± 0.36	0.001*
Serum Sodium (mmol/L) (Mean ± SD)	140.5 ± 3.1	138.3 ± 2.5	0.004*	141.7 ± 2.7	140.13 ± 1.96	0.001*	140.6 ± 1.96	140.6 ± 2.47	0.001*
Serum Potassium (mmol/L) (Mean ± SD)	4.3 ± 0.34	4.26 ± 0.26	0.52**	4.3 ± 0.38	4.9 ± 0.53	0.001*	4.26 ± 0.34	4.8 ± 0.5	0.001*

*Significant, **Not significant

Table-II: Frequency of nephrotoxicity in all the groups.

Histo-pathology	Frequency (%)		
	Group A	Group B	Group C
Normal	10 (100%)	-	-
Minimal	-	-	7 (23%)
Mild	-	1 (3%)	19 (63%)
Moderate	-	2 (6%)	2 (6%)
Marked	-	27 (90%)	2 (6%)

DISCUSSION

MRSA and other difficult to treat infections have been a source of concern for the clinicians. Vancomycin has turned out to be a mainstay to curb these infections. One constraint which can confine the use of vancomycin is the development of nephrotoxicity[16]. Current animal data suggests that vancomycin induces renal proximal tubular epithelial cells ischemia, apoptosis, tubular dilatation and interstitial edema. Generation of the reactive oxygen radicals with oxidative phosphorylation is the most probable mechanism for vancomycin induced nephrotoxicity[17].

In our study, vancomycin administration triggered, raised serum urea and creatinine levels, as an evidence of modification of kidney function. Similarly the serum sodium and potassium levels were elevated too. The damage to the renal parenchymal tissues was evident due to the generation of free radicals and imparting oxidative stress causing diminished antioxidant enzymes activity. To curb this effect of vancomycin, the use of an antioxidant, vitamin E, was explored in our study. Vitamin E is an essential nutrient and a versatile antioxidant, offering substantial protection against various diseases involving oxidative damage. It enhances the survival of renal tubular cells by diminishing apoptosis and necrosis by the virtue of extensive antioxidant activity[18].

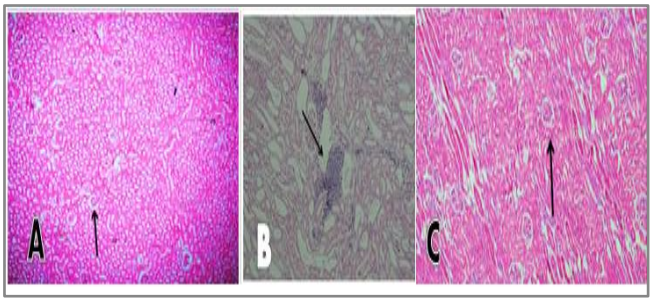

Figure: Normal renal morphology (Group A), Markednecrosis alongwith inflammation (Group B), Almost normal renal architecture (Group C).

In the present study, vitamin E offered ample renal protection induced by vancomycin when it was administered at a dose of 500mg/kg/day for seven days prior to the administration of vancomycin. It caused reversal of histological alterations and functional markers with significant decrease in serum urea and creatinine levels with $p<0.001$. The outcome of the study performed by Derakhshanfar *et al*, was in corroboration to our study[19]. Similar study carried out in rats by Naghibi *et al*, to verify the protection offered by alpha tocopherol against VCM induced nephrotoxicity substantiated our findings[20]. Vitamin E attenuated the toxicity and significantly decreased the serum urea and creatinine levels. Ocak and his fellows, elucidated the effectiveness of vitamin E in preventing VCM induced tubular damage[21]. In another study Vitamin E renal protective role was clearly affirmed in Lithium induced nephrotoxicity. Lithium caused histological damage and biochemical changes by oxidative stress, which were curtailed by Vitamin E administration[22]. In 2017, Seval Yılmaz, and collegeaues, established that Vitamin E offers renal protection against Alfatoxin with improved renal morphology which supports our results[23]. Our study results resemble those demonstrated by Yadav G and his fellow researchers. They drew similar conclusion by proving that Vitamin E offers renal protection[24]. Both the tested drugs shifted histopathological grading from marked inflammation to minimal inflammation in almost all renal cross sections.

Technical Report of the Study

In this study the animal model selected is rabbit while in most of the previous studies rats or mice were used. The dose of Vitamin E is also different. We selected 500mg as compared to the 200mg in a different study as the animal models are different in both the studies. The drugs used in this study were purchased from local market manufactured by local pharmaceutical industries. This enabled us to study the efficacy of our local brands. In future we can focus on the bio equivalence studies on our local drugs used in this study.

CONCLUSION

Bearing in mind the oxidative stress, being the most important aspect in the development of nephrotoxicity due to vancomycin, Vitamin E was used in this study as it protects against the oxidative stress associated with vancomycin induced nephrotoxicity. Further exploration is required to establish the precise dose and protective mechanism in human beings.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any authors.

REFERENCES

1. Filippone EJ, Kraft WK, Farber JL. The nephrotoxicity of vancomycin. Clin Pharmacol Ther 2017; 102(3): 459-69.
2. Van Hal SJ, Paterson DL, Lodise TP. Systematic review and meta-analysis of vancomycin-induced nephrotoxicity associated with dosing schedules that maintain troughs between 15 and 20 milligrams per liter. Antimicrob Agents Chemother 2013; 57(5): 734-44.
3. Mergenhagen KA, Borton AR. Vancomycin nephrotoxicity: a review. J Pharmacy Pract 2014; 27(6): 545-53.
4. Elyasi S, Khalili H, Dashti-Khavidaki S, Mohammadpour A. Vancomycin-induced nephrotoxicity: mechanism, incidence, risk factors and special populations. A literature review. Eur J Clin Pharmacol 2012; 68(9): 1243-55.
5. Takigawa M, Masutomi H, Kishimoto Y, Shimazaki Y, Hamano Y, Kondo Y, et al. Time-Dependent Alterations of Vancomycin-Induced Nephrotoxicity in Mice. Biol Pharmaceutical Bulletin 2017; 40(1): 975-83.
6. Celik I, Cihangiroglu M, Ilhan N, Akpolat N, Akbulut HH. Protective effects of different antioxidants and amrinone on vancomycin-induced nephrotoxicity. Basic Clin Pharmacol Toxicol 2005; 97(5): 325-32.
7. Liu P, Feng Y, Wang Y, Zhou Y, Zhao L. Protective effect of vitamin E against acute kidney injury Bio-Med Materials Enginer 2015; 26(1): S2133-44.
8. Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. Free Radical Biol Med 2007; 43(1): 4-15.
9. Stojiljkovic N, Ilic S, Veljkovic M, Todorovic J. Effects of supplementation with vitamin e on gentamycin-induced acute renal failure in rats. FactaUniversitatis, Series: Med Biol 2014; 16(2): 61-66.
10. Kara A, Cetin H, Oktem F, Metin Ciris I, Altuntas I, Kaya S. Amikacin induced renal damage and the role of the antioxidants on neonatal rats. Renal Failure 2016; 38(5): 671-77.
11. Ghlissi Z, Hakim A, Mnif H, Zeghal K, Rebai T, Sahnoun Z. Evaluation of the Protective Effect of Vitamins E and C on Acute Tubular Damage Induced by Colistin in Rat Model. Am J Phytomed Clin Ther 2015; 3(1): 43-53.
12. Cuce G, Esen HH, Koc T, Canbaz HT, Limandal C, Kalkan S, et al. Vitamin E partially ameliorates cyclophosphamide-induced nephrotoxicity in rats. Progress Nutr 2016; 18(2):140-05.
13. Sadeeshkumar V, Arul D, Ravichandran S. Protective effects of gallic acid in the renal markers, histopathology and immunohistochemical studies on vancomycin induced nephrotoxic rats. Int J Adv Life Sci 2013; 6(3): 356-64.

14. Hemati S, Jolfaie NA, Gookizadeh A, Rafienia M. The effects of vitamin E and selenium on cisplatin-induced nephrotoxicity in cancer patients treated with cisplatin-based chemotherapy: A randomized, placebo-controlled study. J Res Med Sci 2012; s49(1): 16-17.
15. Goering PL, Fisher BR, Noren BT, Papaconstantinou A, Rojko JL, Marler RJ. Mercury induces regional and cell-specific stress protein expression in rat kidney. Toxicol Sci 2000; 53(1): 447-57
16. Bamgbola O. Review of vancomycin-induced renal toxicity: an update. Ther Adv Endocrinol Metabol 2016; 7(3):136-47.
17. Lodise TP, Lomaestro B, Graves J, Drusano GL. Larger Vancomycin Doses (at Least Four Grams per Day) Are Associated with an Increased Incidence of Nephrotoxicity. Antimicrob Agents Chemother 2008; 52(4): 1330-36.
18. Darwish MA, Abo Youssef AM, Khalaf MM, Abo Saif AA, Saleh IG, Abdelghany TM. Vitamin E mitigates cisplatin induced nephrotoxicity due to reversal of oxidative/nitrosative stress, suppression of inflammation and reduction of total renal platinum accumulation. J Biochem Molecul Toxicol 2017; 31(1): 1-9.
19. Derakhshanfar A, Bidadkosh A, Sadeghian MH. L-methionine attenuates gentamicin nephrotoxicity in male Wistar rat: pathological and biochemical finding. Iran J Veterinary Res 2009; 10(4): 323-28.
20. Naghibi B, Ghafghazi T, Hajhashemi V, Talebi A, Taheri D. The effect of vitamin E in prevention of vancomycin-induced nephrotoxicity in rats. Res Pharmaceut Sci 2007; 1(1): 104-11.
21. Ocak S, Gorur S, Hakverdi S, Celik S, Erdogan S. Protective effects of caffeic acid phenethyl ester, vitamin C, vitamin E and Nacetylcysteine on vancomycininduced nephrotoxicity in rats. Basic Clin Pharmacol Toxicol 2007; 100(5): 328-33
22. Omar HE, Ibrahim AT, Magdy MA, Ahmed EA. The protective effects of zinc and vitamin E supplementation against kidney toxicity by lithium in rats. Eur J Biol Res 2016; 6(1):21-7.
23. Yılmaz S, Kaya E, Comakli S. Vitamin E (α tocopherol) attenuates toxicity and oxidative stress induced by aflatoxin in rats. Advances in clinical and experimental medicine: official organ Wroclaw Med Uni 2017; 26(6): 907-17.
24. Yadav G, Awasthi J R, Pandey N, Shrestha S, Jha CB. The effect of vitamin E in the gentamicin induced nephrotoxicity in wistar albino rats-a histological perspective. Int J Ther App 2017; 34(1): 7-10.