Alpha-tocopherol ameliorates nephrotoxicity associated with the use of colistin in rabbits

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Abstract: The alarming rise in the rate of multi drug resistant, life threatening gram negative infections has brought renaissance in the use of Colistin for last two decades. The major constraint in its utilization is its nephrotoxicity. Therefore it is being underused which is favoring the development of resistance. This study assesses the prevention of nephrotoxicity associated with high and low toxic doses of Colistin by alpha-tocopherol. Thirty rabbits were randomly divided into five groups. Baseline serum urea, creatinine and electrolytes were estimated. A loading dose of colistin was given in the form of infusion followed by I.M injections for six days. In the preventive groups α-tocopherol was additionally given orally for two weeks. Rabbits were sacrificed 24 hours after the last dose. The kidney slides graded and statistically analyzed using "chi square". The results of serum analysis were compared using one way analysis of variance followed by post hoc tukey test. There was marked nephrotoxicity in high toxic group where as in low toxic group mild nephrotoxicity was evident. Alpha-tocopherol attenuated the renal insult in both the toxic groups. As damage induced by colistin is oxidative in nature, thus it was concluded that the protection offered by α- tocopherol is due to its antioxidant activity.

Keywords: Colistin, Alpha-tocopherol, Nephrotoxicity, Oxidative renal damage, Colistin methanesulfonate sodium.

INTRODUCTION

The emergence of life threatening multidrug resistant (MDR) Gram-negative bacterial infections lead to the resurgence in the use of Colistin, a polymyxin E; which was once rejected due to its high potential to cause nephrotoxicity (Giamarellou, 2010). Colistinis a bactericidal antibiotic that now constitutes the salvage therapy of patients of ventilator-associated pneumonia (VAP), gram-negative septicemia and nosocomial infections in neutropenic hosts (Vicari *et al.,* 2013).

The decline in the rate and seriousness of renal complication reported currently is attributed to the IV use of the prodrug, colistin methanesulfonate sodium (CMS) instead of colistin sulfate, more purified formulation of CMS, better care and facilities in the ICUs ensuring prompt management and maintenance of fluid and electrolyte balance and avoidance of administration of concurrent nephrotoxins (Spapen *et al.,* 2011)

How colistin produces the renal insult is still under active investigation. Studies on animal models conclude that the potential to cause AKI (acute kidney injury) depends on the total dose and duration of CMS therapy (Biswas *et al.,* 2012).The nephrotoxicant potential exacerbates with dose escalation. This is supported by in vitro studies which demonstrate that toxicity of colistin on mammalian urothelium is concentration and time dependent (Ma *et al.,* 2009). Colistin does not lead to permanent kidney damage; however the deteriorating renal functions contain an independent risk of mortality in a critically ill patient (Ko *et al.,* 2011). Hypothetically the D-amino acid and fatty acid molecules in the structure of colistin produce the injury (Falagas *et al.,* 2006). Being a cation with high lipid affinity, it binds to the phospholipids of the kidney tissue increasing membrane permeability, separating tubular cells, lysis and apoptosis (Ma *et al.,* 2009). Colistin binding with the tissue may generate superoxide radicals producing the actual renal insult as evidenced by contemporary studies designed on animal models (Ozyilmaz *et al.,* 2011). In these studies different antioxidants like melatonin (Yousef *et al.,* 2011a), N-acetylcysteine (Ozyilmaz *et al.,* 2011) and ascorbic acid (Yousef *et al.,* 2011 b) have been successfully used to ameliorate the nephrotoxicity due to use of colistin.

Alpha-tocopherol is the most common and biologically active form of vitamin E (Engin, 2009). It is very efficient, highly tolerable and cost effective natural antioxidant (Feng*et al.,* 2010). It maintains the integrity of the biological membranes in plasma, red blood cells and tissues (Pamukcu *et al.,* 2011). The antioxidant ability is attributed to the free hydroxyl groups on the aromatic ring (Mohamad *et al.,* 2012).

Our study aims to analyze the nephrotoxicity associated with low and high doses of colistin in rabbits and the nephroprotection offered by alpha-tocopherol.

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MATERIALS AND METHODS

The laboratory based randomized controlled trials were held in the animal house of the department of Pharmacology & Therapeutics, Army Medical College; Rawalpindi. The study was approved from the Ethics committee of "Centre for Research in Experimental and applied Medicine" Army Medical College. Thirty healthy adult White New Zealand rabbits of both sexes weighing about 2 to 2.5kg were randomly assigned into five groups. Standard laboratory conditions were maintained and their diet consisted of carrots, turnips, peas, grams and tap water *ad libitum.* The study period consisted of fifteen days after the one week period for acclimatization (Ahmed *et al*., 2015).

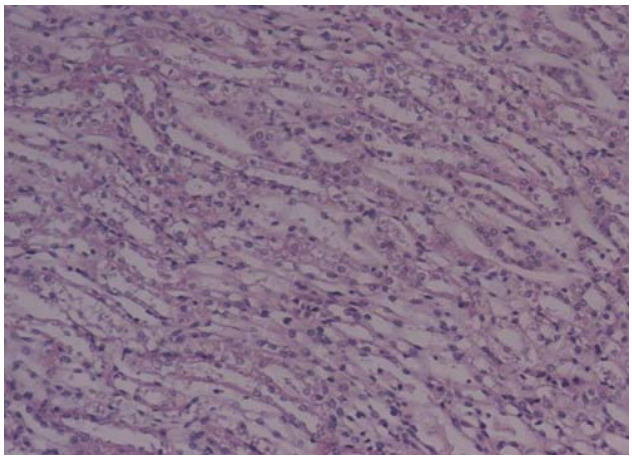

Fig. 1: Microscopic structure of the renal cortex of a rabbit from group A showing normal histology. (X200)

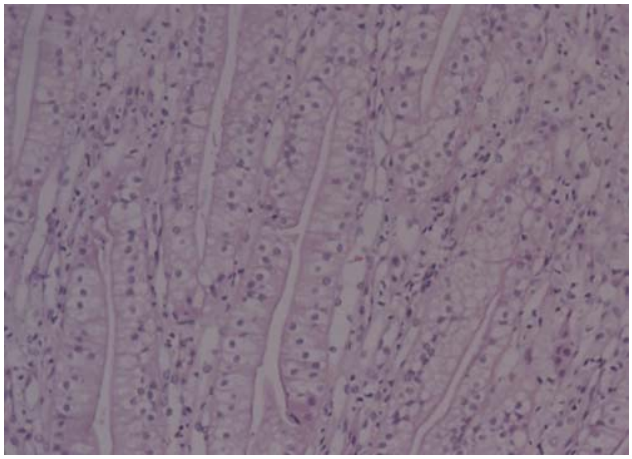

Fig. 2: Microscopic structure of the renal cortex of a rabbit from group B showing moderate nephrotoxicity (grade 2 necrosis). (X200). This is evident from necrosis of the epithelial cells.

Weights of animals were recorded and blood samples collected twice; on the first and last day of study. Before the actual study a preliminary project was carried out to establish the low and high nephrotoxic doses of colistin in rabbits by histopathology analysis. In this pilot project multiple groups of three rabbits were used to formulate a proper methodology for the study. According to this loading dose of colistin must be administered as slow intravenous infusion to avoid respiratory embarrassment caused by high doses of this drug.

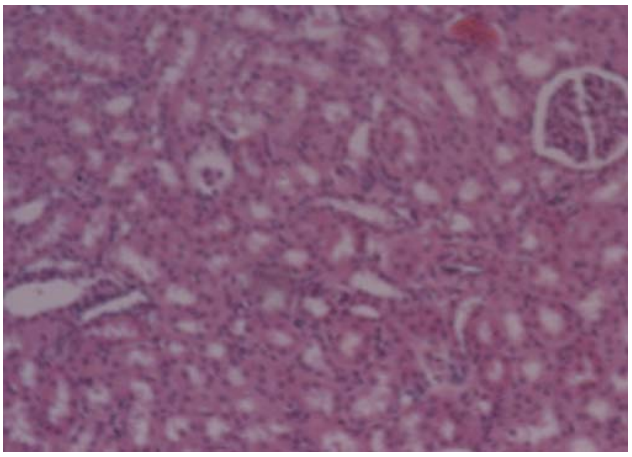

Fig. 3: Microscopic structure of the renal cortex of a rabbit from group C showing mild nephrotoxicity (grade 1 necrosis). (X200). This is evident from tubular dilatation and prominent nuclei

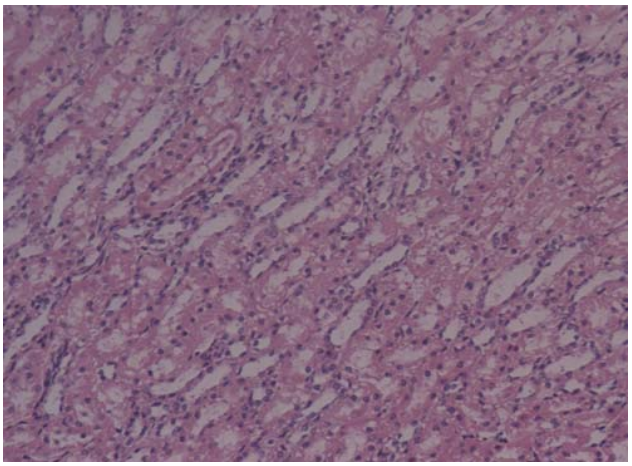

Fig. 4: Microscopic structure of the renal cortex of a rabbit from group D showing mild nephrotoxicity (grade 1 necrosis shown by dilated tubules). (X200).

The control group A received normal saline 1ml per oral (PO) daily. On 8^{th} day 25ml normal saline intravenous infusion over two to three hours was given and on the last six days 1ml normal saline intramuscularly was administered.

Group B (high toxic) received 1ml P.O. saline every day, on eighth day 120mg colistin methatesulfonate sodium (CMS) in 25ml normal saline I.V. infusion over two and half hours as loading dose was administered and then 30 mg colistin sulfate I/M for last six days.

Table 1: Effects of High Dose (120 mg/Kg) alone (group B) and with α-Tocopherol (group D); Low Dose (80 mg/Kg) alone (group C) and with α-Tocopherol (group E); Colistin on Renal Functions of Rabbits[1]

Serum analysis

TESTS	Group A	Group B	Group C	Group D	Group E	Anova
Serum urea (mmol/L) DAY-0	7.45± 0.9	5.10±0.8	10.1±1.4	8.2±0.3	8.8±1.1	0.022*
DAY-15	6.61±1.1	11.5±0.2	13.6±1.9	9.8± 1.2	8.1±0.9	0.531
P value	0.16	0.0001*	0.01*	0.10	0.30	
Serum Creatinine (µmol/L) DAY-0	80.0±6.49	75.5±5.4	83.8±3.3	70±2.8	71.5±5.1	0.254
DAY-15	64.1±8.9	128.6±8.2	103.3±3.5	80±10	79.5±9.1	0.000*
P value	0.11	0.003*	0.007*	0.116	0.28	
Serum Sodium (mmol/L) DAY-0	134.8±1.1	134.6±0.7	135.1±1.04	140±0.7	136±2.4	0.045*
DAY-15	134.3±0.66	132.5±1.38	136.1±2.4	130±3.3	137±0.8	0.163*
P value	0.29	0.14	0.3	0.029*	0.30	
Serum potassium (mmol/L) DAY-0	4.6±0.33	4.0±0.16	3.8±0.24	4.2±0.2	4.7±0.4	0.128
DAY-15	4.5±0.22	5.2±0.11	5.6±0.35	6.2±0.2	5.9±0.2	0.000*
P value	0.33	0.001*	6.0	0.000*	0.040*	

[1]n = 6, Results are expressed as mean ± SEM (standard error of mean).
P value <0.05 = Significant (*)
P value >0.05 = Non Significant (Ns)

Table 2: Post Hoc Comparisons signifying the Effects of High Dose (120mg/Kg) alone (group B) and with α-Tocopherol (group D); And Low Dose (80mg/Kg) alone (group C) and with α-Tocopherol (group E); Colistin on Renal Functions of Rabbits

Groups	A				B			C		D
Serum Analysis	B	C	D	E	C	D	E	D	E	E
Urea mmol/L	0.98	0.93	0.99	0.45	0.99	1.0	0.7	0.9	0.8	0.6
Creatinine µmol/L	0.0	0.2	0.6	0.6	0.2	0.00	0.0	0.3	0.2	1.0
Sodium mmol/L	0.9	0.9	0.7	0.7	0.7	0.9	0.4	0.3	0.9	0.1
Potassium mmol/L	0.3	0.02	0.00	0.00	0.6	0.03	0.2	0.3	0.9	0.8

RESULTS

The animals in the group B were reluctant to feed in the second week of the study period. Some of them were dehydrated, weak and isolated. Rabbits in all the other groups consumed normal diet with an adequate intake of water.

The animals in the Group A significantly gained weight, 1.4±0.005% with $p<0.04$ for group A. There was statistically significant weight loss in Group B. The results for serum urea, creatinine and electrolytes are summarized and compared in tables 1& 2.

DISCUSSION

In our study, alpha-tocopherol 200mg orally daily showed complete nephroprotection when administered for one week before exposing the animals to colistin and in the subsequent week half an hour prior to colistin treatment in both the diverse dose groups. In terms of safety, the dose used i.e. 200 mg was found to be non-toxic to rabbits (Lebas, 2010). The NOAEL (No observable adverse effects level) with α-tocopherol is 643 mg/kg (Mohamad*et al.,* 2012). Alpha-tocopherol when given to rodents by oral route for one week increases antioxidant defenses in the kidneys (Patra *et al.,* 2001). This has also been demonstrated in rabbits by Li *et al.,* 2004 who added α-tocopherol in the feed of rabbits and confirmed an increase in antioxidant activity in various body tissues. Maximum plasma levels of α-tocopherol are achieved in 4-6 hrs (Mustacich *et al.*, 2007). Therefore, we administered α-tocopherol prior to start of colistin therapy to ensure adequate serum levels of the agent by the time formed colistin levels are maintained in four to seven hours. This also minimized any possible interaction during administration of the two drugs. There was a statistically significant decrease of the serum urea ($p< 0.02$), creatinine ($p<0.004$), and attenuation of the pathological lesions in the kidneys ($p<0.01$) with majority of the slides falling in the normal architectural pattern in the low dose α-tocopherol group. However, in high dose α-tocopherol group the fall in creatinine was noteworthy ($p<0.001$) and histological examination also revealed reduction of the insult as evident in the positive control ($p<0.04$). Serum urea was not altered significantly in this preventive group. Nonetheless, changes in serum urea alone do not conclusively indicate renal status

(Jayasundera and Macnab, 2012) and must be analyzed in relation to other parameters, say creatinine in case of this study.

A correlation between development of nephrotoxicity due to colistin and the progression of oxidative stresses has been well-demonstrated by Ozyilmaz *et al.,* 2011 using rat as experimental animal model. They certified the increase in renal tissue superoxide dismutase (SOD) levels, malondialdehyde (MDA) activity, inducible nitric oxide syntheses and neurotrophin-3 on immunocyto chemical staining with the use of colistin. These changes were reversed with the concomitant use of N-Acetyl cysteine, an antioxidant. Thus implicating that colistin induced renal offense may be attributed to oxidative stress. Similarly, Yousef *et al.,* (2011a) employed melatonin to abate the nephrotoxicity caused by colistin. In their study, they achieved both biochemical and histological renal protection in rats. In another series of experiment, Yousef *et al.,* (2011 b) demonstrated that co-administration of high dose ascorbic acid protects against colistin induced apoptosis in the rat renal proximal tubular cells. Ascorbic acid (Riabchenko *et al.,* 2010) and melatonin (Tomas-Zapico and Coto-Montis, 2007; Reiter *et al.,* 2010) are both very efficient free radical scavengers. This strongly suggests a key role of reactive oxygen species and highlights our prospect of using an antioxidant for the aversion of colistin-induced renal injury to widen the therapeutic window.

CONCLUSION

Thus we conclude that pre-treatment with α-tocopherol abates the renal injury associated with the use of colistin. Clinical trials could be carried out to check whether α-tocopherol offers similar level of protection in humans against the nephrotoxicity provoked by colistin.

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