ESTABLISHMENT AND COMPARISON OF NEPHROTOXICITY ASSOCIATED WITH THE USE OF VARIOUS DOSES OF COLISTIN IN RABBITS

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ABSTRACT

Objective: This study was designed to assess the nephrotoxicity associated with various doses of Colistin sulfate in rabbits.

***Study Design*:** Laboratory based randomized controlled trials,

Place and Duration of Study: This study was held at Army Medical College, Rawalpindi. Study period was from 15th April till 30th April 2012.

Material and Methods: Rabbits were divided into three groups of six rabbits each. Baseline serum urea, serum creatinine and serum electrolytes were estimated. A loading dose of colistin infusion was given followed by I.M injections for six days. Rabbits were sacrificed 24 hours after the last dose and both kidneys were sent for histopathology.

Results: There was marked nephrotoxicity in high toxic group where as in low toxic group mild nephrotoxicity was evident.

Conclusion: It was established that we may safely escalate dose of colistin up to four times the currently recommended schedule to combat the threat of resistance when using it for one to two weeks.

Keywords: Colistin, Colistin methanesulfonate, Nephrotoxicity.

INTRODUCTION

Nosocomial infections pose a huge threat to the safety of the indoor patients; being the sixth leading cause of death in the western world[1]. These statistics further rise in our part of the globe enormously increasing the cost of health care[2]. The causative organisms are the notorious gram-negative superbugs that possess the ability of mutating and hence are resistant to a number of drugs[3]. The catastrophe is further aggravated by the inadequate drug discovery due to enormous cost and less redeems[4]. As the pharmaceutical pipeline is waning, these Multidrug resistant gram-negative organisms have left us with no option than to resort to much older, toxic but potent and effective antibiotic, colistin[5].

Colistin is a bactericidal antibiotic that constitutes the salvage therapy of patients of ventilator-associated pneumonia (VAP), gram-negative septicemia and nosocomial infections in neutropenic hosts[6]. It is produced from a sub species colistinus of Bacillus Polymyxavar[7]. Use of colistin waxed and waned with time due to its high potential to cause nephrotoxicity[8]. It was the high rates and fatal consequences of this effect that led to rejection of the systemic use of colistin. In the literature reported after the resumption however, its incidence ranges from 6% to 55%[9]. This lack of accurate statistical data and for the dread of causing insult to kidneys; physicians are under-using it. This is favoring the development of resistance and we are at the verge of losing a wonderful drug[10].

The study aims to analyze the extent of nephrotoxicity associated with low and high doses of colistin in rabbits and therefore determine the safe dose escalation to combat resistance associated with lower doses.

MATERIAL AND METHODS

These laboratory based randomized controlled trials were conducted in the animal house of the department of Pharmacology & Therapeutics, Army Medical (AM) College;

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Rawalpindi from 2nd-15th May 2012. The approval for the study was sought from the Ethics committee of "Centre for Research in Experimental and applied Medicine (CREAM)" Army Medical College.

Eighteen healthy adult rabbits including both males and females weighing about 1 to 2 kg of mixed breed were included in the study and randomly divided into three groups through random numbers. Standard laboratory conditions were maintained in animal house. Rabbits were acclimatized in the preliminary week[11]. They were fed on same standard diet and tap water ad libitum for drinking.

Following chemicals were used for the study.

Colistin sulfate was purchased as a substance of pharmaceutical grade from Merck Pharmaceuticals, Islamabad.

The commercial formulation colistin methanesulfonate sodium (CMS) which is used for systemic applications[12] prepared by Forest laboratories, United Kingdom (Colomycin) was imported. Rabbits were weighed prior to the first dose on day one and then 24 hours after the last dose, just before sacrificing them. Blood samples were collected by venipuncture of the auricular marginal vein[13]; on the first and last day of study before sacrificing the animals.

The dose design of our study was based on the preliminary studies because to our knowledge this is the first study that substantiated the use of rabbit as a model for colistin/CMS nephrotoxicity. In these set of experiments we studied three groups with three rabbits each by administering 10, 20 and 30mg of colistin sulfate /kg body weight for seven days. The loading dose was given in intravenous infusion form due to the high incidence of neurotoxicity at these doses.

Table-1: Effects of high dose (120 mg/Kg) group B; and low dose (80 mg/Kg) group C; colistin on renal functions of rabbits serum analysis:

Tests	Group A	Group B	Group C	Anova
Serum urea (mmol/L) DAY-0	7.45 + 0.9	5.10 + 0.8	10.1+1.4	0.001*
Day-8	6.61+ 1.1	11.5+ 0.2	13.6+1.9	0.001*
p -value	0.16	0.0001*	0.01*	
Serum creatinine (µmol/L) DAY-0	80.0 + 6.49	75.5+5.4	83.8+3.3	0.047*
Day-8	64.1+8.9	128.6 + 8.2	103.3+3.5	0.001*
p value	0.11	0.003*	0.007*	
Serum Sodium (mmol/L) Day-0	134.8 + 1.1	134.6 + 0.7	135.1+1.04	0.67
Day-8	134.3 + 0.66	132.5+1.38	136.1+2.4	0.006*
p -value	0.29	0.14	0.3	
Serum potassium (mmol/L) DAY-0	4.6 + 0.33	4.0 + 0.16	3.8 + 0.24	0.001
Day-8	4.5 + 0.22	5.2 + 0.11	5.6+0.35	0.001*
p -value	0.33	0.001*	6.0	

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

The rabbits (n=6) in different experimental groups were given colistin sulfate, CMS, once daily according to the following schedule.

Group-A: It was the control group and received 25ml normal saline intravenous infusion over two to three hours on the first day and for the next six days 1ml normal saline intramuscularly was administered.

Group-B: Group B received 120mg colistin methanesulfonate sodium (CMS) per kg body weight in 25ml normal saline I.V. infusion over two and half hours as loading dose was

administered and then 30 mg colistin sulfate/kg body weight I/M for next six days. The animals were sacrificed on eighth day. This served as high toxic group.

Group-C: Group C was low toxic group and received 80 mg of CMS infusion per kg body weight and then 10 mg Colistin/kg body weight I/M for last six days.

Twenty four hours after the last dose of the drug rabbits were sacrificed and kidneys were prepared for histological examination. The kidney slides were examined under light microscope and were graded as follows[14,15].

Grade 0: No significant change.

Grade I: Mild damage; indicated by mild acute tubular damage with tubular dilatation, prominent nuclei and a few pale tubular casts.

Grade II: Moderate damage; signified by severe acute tubular damage with necrosis of tubular epithelial cells and numerous tubular casts.

Grade III: Severe damage; evident from acute cortical necrosis/infarction of tubules and glomeruli with and without papillary necrosis. Data analysis the statistical analysis of the results obtained from all set of experiments was prepared on computer using SPSS 16 application. One way analysis of variance (ANOVA)was applied followed by 'Post Hoc Tukey' test to find out the differences between various means. The difference was considered significant for a p value of 0.05 or less. Within group comparisons are done by applying paired sample't' test. The histopathology slides were graded and comparison of the results of various parameters was done using 'Chi Square.'

RESULTS

There was no mortality in the animals of actual study group. The animals in the group B were reluctant to feed in the second week of the study period. Some of them were also found 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 in tables-1 and table-2.

Table-2: Post Hoc comparisons signifying the effects of high dose (120 mg/Kg) group B; and low dose (80 mg/Kg) group C; colistin on renal functions of rabbits.

Serum Analysis	Group	Group	Mean Difference	Std. Error+	*p*-value
Serum urea mmol/L	A	B	4.95	1.88	0.04
		C	7.05	1.88	0.00
	B	C	2.10	1.88	0.51
Creatinine µmol/L	A	B	64.50	10.33	0.00
		C	39.16	10.33	0.00
	B	C	25.33	10.33	0.06
Sodium mmol/L	A	B	1.83	2.35	0.72
		C	1.83	2.35	0.72
	B	C	3.66	2.35	0.29
Potassium mmol/L	A	B	0.66	0.35	0.18
		C	1.11	0.35	0.01
	B	C	0.45	0.35	0.43

[1]n = 6, Results are expressed as mean + SEM (standard error of mean).

In summary, colistin administration in low toxic doses leads to moderate nephrotoxicity whereas high toxic doses lead to severe nephrotoxicity. This was evident by rising serum urea, serum creatinine and serum potassium levels and increasing necrosis and apoptotic changes on histopathological examination. There was no change in serum sodium levels.

DISCUSSION

In our study we found that by increasing the colistin dose up to 6 to 12 times that of the currently recommended regimen for one week led to moderately severe renal disease. In the low toxic dose group we found statistically significant elevation of the serum urea, creatinine and potassium with mild to moderate renal pathological changes. Similarly in the high toxic group the rise in the serum urea, creatinine and potassium was significantly more than that in the low toxic group and moderate to severe nephrotoxicity on the histopathology. This group also showed a significant mean weight loss of 12% in the animals at the end of the study period. Wallace et al. reported mild histopathological changes in rat kidneys exposed to 20 mg/kg/8 hrs for one week, moderate nephrotoxicity with a dose of 30 mg/kg/12 hrs whereas severe renal pathological insult with a dose of 150mg/kg/12 hrs[16] which is consistent with our results as both our dose groups lie between 30 and 150 mg range. Yousefet al., in two consecutive studies on rats also described severe acute tubular damage with necrosis and apoptosis of the epithelial cells by a cumulative colistin sulfate dose of 36.5 mg. They also documented the increase in serum creatinine levels resembling our results[14,15]. Further to strengthen this line of reasoning a study conducted to validate the oxidative stress imposed by the use of colistin in various tissues of the body demonstrated an increase in the serum urea and creatinine and decrease in creatinine clearance with a dose of 25 mg CMS i.p. for six days[17]. Clinical studies in recent years have also graded colistin induced nephrotoxicity to be of moderate nature on the basis of RIFLE criteria as compared to that described in the previous literature[18-21]. A recent prospective, observational, cohort study conducted by Dalfinoet al., also concludes that the extended-interval high dose CMS regimen is highly efficacious without severe renal toxicity[22]. Clinical studies that evaluated nephrotoxicity and its consequent mortality rates between various other groups of antibiotics possessing potential for renal injury with colistin have also shown no statistically significant increase in these parameters with the use of Colistin[23-25]. It is worth mentioning here that breakthrough of aminoglycosides lead to the discontinuation of colistin use because they were previously thought to be less nephrotoxic, are now known to exhibit similar and even somewhat higher nephrotoxic potential than colistin. Goundenet al., demonstrated this in a clinical retrospective cohort study in which tobramycin was compared to colistin in terms of effectiveness and safety[26]. We studied electrolytes and weight changes as well. To our knowledge no other experimental study on animal models has shown an increase in serum potassium and decrease in weight with the use of colistin. The increase in serum potassium indicates ensuing acidosis whereas weight loss was a consequence of reluctance to oral intake may be induced by acidosis and impending renal failure.

CONCLUSION

It was established that we may safely escalate dose of colistin up to four times the currently recommended schedule to combat the threat of resistance when using it for one to two weeks.

CONFLICT OF INTEREST

This study has no conflict of interest to declare. Abstract and results of this study were accepted and presented in an oral presentationat the International conference on Medical Education, organised by Association for Excellence in Medical Education (AEME) and held on 7th-9th March 2014 at UHS.

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