STUDY OF PREVENTIVE EFFECT OF MELATONIN ON HIGH DOSE VANCOMYCIN INDUCED NEPHROTOXICITY IN RABBITS

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ABSTRACT

Objective: To explore the nephropreventive effects of melatonin on vancomycin-induced nephrotoxicity in rabbits.

Study Design: Laboratory based randomized controlled trial.

Place and Duration of Study: Department of Pharmacology and Therapeutics, Army Medical College, Rawalpindi, from May 2017 to June 2017.

Material and Methods: Seventy rabbits were divided into three groups. Group A served as a control group (n=10), group B (n=30) received I/P vancomycin 200mg/kg twice a day for seven days while group C (n=30) received I/P melatonin 10mg/kg 30 minutes prior to vancomycin administration for seven days. Animals were sacrificed on the eighth day. Biochemical analysis was done for serum urea, creatinine, sodium and potassium on day 0 and day 8. Kidneys were sent for histopathology. Statistical analysis was carried out by using Microsoft Office Excel 2010 and SPSS version 21. One way ANOVA, followed by 'Post Hoc Tukey' test was used for biochemical parameters.

Results: Vancomycin induced massive renal damage (grade III) and led to elevation in biochemical parameters, while melatonin pretreatment prevented the renal damage and the biochemical parameters were also significantly reduced with a *p*-value of <0.001 for serum urea, creatinine and potassium but had insignificant *p*-value for serum sodium levels.

Conclusion: The study outcome indicates the potential of melatonin to prevent Vancomycin induced nephrotoxicity by virtue of its antioxidant property.

Keywords: Melatonin, Nephrotoxicity, Vancomycin.

INTRODUCTION

Vancomycin, a glycopeptide antibiotic, has been considered as a first line drug to treat serious gram-positive infections involving methicillin-resistant *Staphylococcus aureus* (MRSA) and enterococcal infections[1]. It turned out to be as a highly recommended agent for difficult to treat infections in the 1970s[2]. Over the next several decades, due to the gradual rise in the incidence of MRSA infections in the community and health care settings, its usage dramatically increased. While newer anti-MRSA medications are good therapeutic alternatives still vancomycin proves to be a better option in all situations[3]. The patients who are administered vancomycin for the treatment of MRSA infections report treatment failures despite in vitro susceptibility. In order to overcome treatment failures the employment of vancomycin doses higher than those approved by the FDA (1g q 12h) are being considered[4]. To maintain the trough concentrations of 10-20 µg/mL, higher doses of vancomycin should be administered as recommended by infectious diseases society of America (IDSA)[5]. A potentially serious side effect associated with vancomycin therapy is nephrotoxicity which impairs the high doses or combination chemotherapy[6]. The prevalence of vancomycin-induced nephrotoxicity (VIN) has been reported to be between 5 and 35%. The proposed pathology of vancomycin induced nephrotoxicity is said to be stimulation of oxygen consumption and elevated cellular adenosine triphosphate concentrations supports oxidative phosphory-

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Received: 30 Mar 2018; revised received: 29 May 2018; accepted: 08 Jun 2018

lation which produces oxygen free radicals leading to the injury and hence oxidative stress, inflammatory events and apoptotic cell death in the renal proximal tubule cells which leads to renal tubular ischemia. There is enhanced cell proliferation as well[7]. The most desirable strategy in the treatment of MRSA infection without effecting its efficacy is the prevention of vancomycin induced nephro-toxicity. Several protective strategies have been recommended to lower vancomycin induced renal proximal tubule damage. Among them antioxidant use has shown promising results. As, antioxidants are considered to play an all important role in preventing the formation of reactive oxygen species and establish the therapeutic basis for preventing or treating acute renal failure in humans[8]. Melatonin, a potent antioxidant, (N-acetyl-5-methoxytriptamine) is synthesized and released into the circulation and especially into cerebrospinal fluid by the pineal gland in a circadian rhythm[9]. Melatonin eliminates H_2O_2 by increasing the activity of enzymes and yields a product N-acetyle-N (2) formyl-5-methoxy-kynuramine which donates two electrons and acts as free radical scavenger. It also neutralizes the most dangerous endogenous reactive species i.e. the *OH. It slows down lipid peroxidation. Furthermore, melatonin exerts protective effect due to its ability to inhibit the production of NO* and to scavenge ONOO and reduce inflammation. Thus melatonin and its metabolite show an equal or greater antioxidant efficacy as compared to other antioxidants[10]. The aim of the study was to assess the nephroprotective role of melatonin pretreatment in vancomycin induced nephrotoxicity. The data provided would help the prescribing authorities and the health care professionals in ameliorating vancomycin induced nephrotoxicity.

MATERIAL AND METHODS

This was a randomized controlled trial. This study was conducted at the Department of Pharmacology & Therapeutics, Army Medical College, Rawalpindi. The ethical approval for this study was sought from ethics committee of centre for research in experimental and applied medicine (CREAM), Army Medical College, Rawalpindi. The total duration of study was eight weeks. The animals were obtained from National institute of health (NIH), Islamabad. They consisted of seventy healthy adult rabbits both male and female in equal number, weighing approximately 1 to 2 kg. Initially they were selected through non-probability convenience method and then divided randomly by lottery method into 03 groups. They were acclimatized for a week atthe animal house of Army Medical College 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. The first group A, served as control (CTL) consisting of ten rabbits, received single intraperitoneal injection of 1 ml normal saline for seven days. Group B (VCM) consisting of thirty rabbits were given vancomycin 200mg/kg intraperitoneally[11] twice a day for seven days which served as a toxic group. While third group C (Melatonin), consisting of thirty rabbits was given melatonin 10mg intraperitoneally[12], 30 minutes prior to vancomycin and every day at the same time for the next seven days. Weight of rabbits was measured every day on digital weighing scale to adjust the dose. Blood samples were collected on day O from the marginal vein and on the 8th day after sacrificing the rabbits. Histopathological examination was carried out on kidneys by preparing the slides. For this purpose the kidneys were dissected out and rinsed with tap water thoroughly. They were carefully split sagittaly and placed in 10% formalin for next 24 hours for fixation. Histopathological abnormalities were scored according to the following criteria[13]

0 = No cell necrosis

1 = Mild, only single cell necrosis in sparse tubules

2 = Moderate, more than one cell involved in sparse tubules

3 = Marked, tubules exhibiting total necrosis in almost every power field

4 = Massive total necrosis

Statistical Analysis

The statistical analysis of all the results was done on computer using Microsoft Office Excel 2007 and SPSS version 21. The results for serum analysis were expressed as means ± standard deviation (Jaykaran, 2010). One way analysis was done using ANOVA followed by 'Post Hoc Tukey' test for multiple comparisons between the groups. The difference was considered significant for a *p*-value of 0.05 or less. The histopathological slides were analyzed by comparing the results by gp* HP cross tabulation.

RESULTS

After administration of vancomycin (200 mg/kg/body wt) to the animals for seven days, analysis of biochemical parameters revealed a significant elevation of serum urea, creatinine, sodium and and potassium as well. The mean serum urea in group A was 4.5 ± 0.49mmol/L, in group B, 7.9 ± 1.3 mmol/L, while in group C, 4.7 ± 0.78mmol/L (p<0.001). The serum creatinine values in group A, was 82.4 ± 7.4mmol/L. Group B, 94.5 ± 6.8mmol/land group C were 73.10 ± 5.5mmol/L (p<0.001). Mean sodium was 138.3 ± 2.5 mmol/Lin group A, 140.1 ±1.9 mmo/L in group B and174.3 ± 182.2 mmol/L in group C (p=0.473). Mean serum potassium value in group A was 4.26 ± 0.26mmo/L, in group B 4.9 ± 0.51mmol/L and in group C4.3 ± 0.35mmol/L (p<0.001). The means ± SD along with the *p*-values of serum urea, screatinine, sodium and potassium of the three groups is given under as table. Tukey post-hoc comparison between groups for serum urea is significant for the two groups A and B (p<0.001) but not significant for group A and C. For serum creatinine the *p*-value was significant for all the three groups A, B and C (p<0.001) while for serum potassium significant values were observed between group A and B with a *p*-value of p<0.001. On histopathological analysis, group A showed normal architecture in the renal tissues with no signs of necrosis (grade 0). The toxic group B which was administered vancomycin presented massive histological changes with severe tubular necrosis, interstitial inflammation and tubular atrophy (grade-III). While the melatonin treated group showed only mild and moderate renal tissue damage and vancomycin induced tubular necrosis was decreased (grade-1) (figure).

Table-I: Comparison of serum electrolyte parameters of group A (n=10), group B (n=30) and group C (n=30).

Electrolyte Parameters	Groups	Mean ± SD	*p*-value
Serum Urea (mmol/L)	Group A	4.5 ± 0.49	<0.001*
	Group B	7.9 ± 1.3	
	Group C	4.7 ± 0.78	
Serum Creatinine (mmol/L)	Group A	82.4 ± 7.4	<0.001*
	Group B	94.5 ± 6.8	
	Group C	73.10 ± 5.5	
Serum Sodium (mmol/L)	Group A	138.3 ± 2.5	0.473**
	Group B	140.1 ± 1.9	
	Group C	174.3 ± 182.2	
Serum Potassium (mmol/L)	Group A	4.26 ± 0.26	<0.001*
	Group B	4.9 ± 0.51	
	Group C	4.3 ± 0.35	

*Significant, **Not significant, Gp *HP Crosstabulation.

Table-II: HP cross-tabulation table: analysis of histopathological results in group A, B and C.

	Normal	Mild	Moderate	Marked	Massive	Total
Group A	10	0	0	0	0	10
Group B	0	0	3	13	14	30
Group C	0	18	11	1	0	30
Total	10	18	14	14	14	70

DISCUSSION

Clinical data has shown that nephrotoxicity is one of the most crucial adverse effects of vancomycin which may hinder its dose and duration of therapy. The renal impairment has greatly influenced the prognosis and has led to the prolonged hospital stay and increased economic burden on the patient and the health-care system[14]. The present research project was initiated to explore adjunct therapy adaptation. It is an established fact that VCM is accumulated in renal cells and is believed to cause nephrotoxicity. The most established mechanism for VCM nephrotoxicity as authenticated in animal studies, can be at least moderately stated to be an enhanced production of reactive oxygen species, oxidative stress with tubular necrosis, interstial inflammation mitochondrial dysfunction and cellular apoptosis[15,16]. The most marked mechanism is stated to be due to oxidative stress which led to the use of melatonin, a putative antioxidant, to attenuate vancomycin induced nephrotoxicity. Several studies have established its role in protecting kidney against oxidative injury induced by gentimycin[17,18], cisplatin[19], adriamycin[20] and colistin[21]. The present study evaluated that the administration of 10mg/ kg/day of melatonin in a rabbit model, can provide protection against vancomycin induced nephrotoxicity. Both serum urea and serum creatinine levels alongwith serum sodium and potassium levels increased considerably in vancomycin treated animals (p<0.001); but somehow, these effects of VCM were ameliorated by melatonin treatment with significant decrease in these biochemical markers (p<0.001) except for insignificant data for serum sodium. The histopathological examination revealed amelioration of the renal tissue damage. These findings suggest the influence of melatonin pretreatment on VCM induced nephrotoxicity. To abate the organ damage, melatonin has a substantial role as a free radical scavenger of hydroxyl radicals, reduces nitrogen oxide levels and subsequently decreases lipid peroxidation, provides mitochondrial protection, enhances a vital antioxidant enzyme glutathione peroxidase[22]. Ulkan, demonstrated that melatonin and colistin administration for 10 days to rats led to the lowering of the serum urea and creatinine levels. The marked histopathalogical changes that were seen in colistin treated group were not evident in the melatonin–colistin group[21]. Their findings support our study. Our results are completely in harmony with the results of Mehrzadi and friends who proved protective effects of melatonin and atorvastatin on GEN induced nephrotoxicity by decline in serum urea, creatinine, renal reactive oxygen species ROS[23]. Furthermore, our study results resemble study outcome of Yildizand colleagues. They demonstrated that melatonin and vitamin C pretreatment against renal ischemia perfusion injury declined the serum urea, creatinine with improved renal parenchymal structure[24]. Recently in 2017, Monera Al aziz demonstrated protective role of melatonin in Adriamycin induced renal

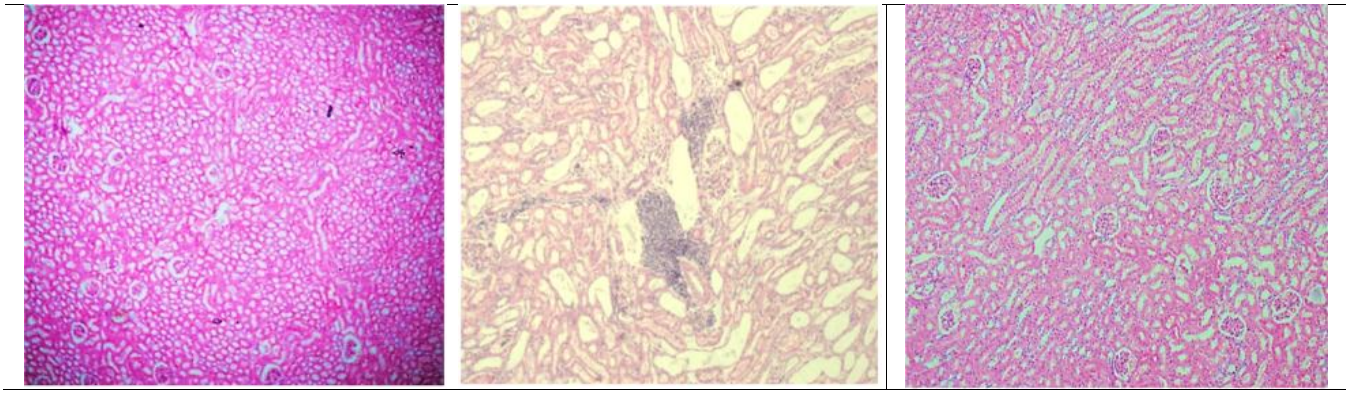

Figure: Normal renal morphology (Group A), Massive necrosis (Group B), Almost normal renal architecture (Group C).

injury[25]. Melatonin administration significantly attenuated the rise in urea, creatinine levels. The findings of the melatonin administration which has a beneficial role in VCM nephrotoxicity proposes the role of reactive oxygen species in renal damage. Therefore our study outlines the need for melatonin use with VCM. In summary, melatonin administration prior to vancomycin attenuated the nephrotoxicity induced by vancomycin. Data suggested significant decrease in serum urea, creatinine, potassium with slight decrease in serum sodium. The histopathological findings showed promising effects on renal parenchymal tissue, with minimal renal tissue damage and protected kidney against oxidative damage.

CONCLUSION

The study outcome indicates the potential of melatonin to prevent Vancomycin induced nephrotoxicity by virtue of its antioxidant property.

CONFLICT OF INTEREST

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

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