ROLE OF SODIUM LOADING AS A REMEDY FOR AMPHOTERICIN B INDUCED NEPHROTOXICITY

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ABSTRACT

Objective: This study was carried out to evaluate the protective role of sodium loading with 0.9% Normal saline against amphotericin B-induced nephrotoxicity.

Study Design: Randomized controlled study.

Place of study and Duration: Pharmacology department and animal house of Army Medical College as part of author's M Phil research project from Feb 2011 to Oct 2011.

Material and Methods: Eighteen rabbits were used in this study. Rabbits were randomly divided into three groups. Each group contained six rabbits. Group A (Control) was injected 10 ml of 0.9% saline intraperitonially. Acute nephrotoxic single dose of amphotericin B, 4 mg/kg was given as intravenous infusion to group B rabbits, group C rabbits received same dose along with prior administration of normal saline, 10 ml/kg intraperitonially for five days. Blood samples were collected from all animals 24 hours after last dose for estimation of blood urea, serum creatinine, serum sodium and serum potassium levels. Histopathology of kidneys was also performed.

Results: Biochemical and histopathological analysis showed significant increase in blood urea and serum creatinine in group B animals that received acute nephrotoxic dose of amphotericin B. However changes in serum electrolytes were not significant. Histopathology showed marked, grade-3 nephrotoxicity. Sodium loading attenuated amphotericin B-induced nephrotoxicity significantly in group C rabbits as their blood urea and serum creatinine levels did not increase significantly in comparison to baseline levels. Histopathology showed mild nephrotoxicity of grade-1, significantly less marked than seen in group B.

Conclusion: Present study concludes that sodium chloride loading significantly reduces the nephrotoxicity of amphotericin B and can be used to mitigate amphotericin B-induced nephrotoxicity.

Keywords: Amphotericin B, Antifungal, Creatinine, Saline, Polyene.

INTRODUCTION

Recent advances in the management of patients with hematological malignancies and transplant recipients have paralleled an increase in the incidence of fungal diseases due to pathogenic genera such as Candida and Aspergillus, and the emergence of less common genera including Fusarium and Zygomycetes[1]. Amphotericin B is an amphoteric polyene macrolide antifungal agent. It has narrow therapeutic window[2]. In many cases, the dose and duration of therapy with conventional amphotericin B preparation is limited by toxicity rather than the clinical status of the patient[3].

Increase in blood urea and serum creatinine is reported in 80% of patients receiving full course of amphotericin B and acute renal failure occurs in one third of patients. The risk of acute renal failure further enhances with increase in cumulative dose[4]. Liposomal preparation reduces the toxicity of amphotericin B[5], but is an expensive option.

A method to reduce undesirable side effects is incorporation of amphotericin B into

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liposomes[6]. Because of its severe nephrotoxicity conventional amphotericin B has been superseded by liposomal formulations in advance and affluent countries however liposomal formulations of amphotericin B are very expensive[7]. Conventional amphotericin B is still extensively used in Pakistan in oncology units, bone marrow and solid organs transplant centers, neutropenic and immunosuppressed patients and in intensive care units. Frequently amphotericin B is administered concomitantly along with other highly nephrotoxic drugs.

Nephrotoxicity of amphotericin B can be minimized by nephroprotective loading with sodium chloride. Sodium chloride loading in rabits prevents the rise in serum creatinine levels during long-term amphotericin B administration that occurs in non-salt supplemented group[8,9]. It is a simple and cost effective method to prevent amphotericin B-induced nephrotoxicity.

MATERIAL AND METHODS

Study was conducted in department of Pharmacology and animal house of Army medical College Rawalpindi, National University of Sciences and Technology Islamabad. Eighteen rabbits purchase from local market were included in this study. Permission was obtained from institutional ethical committee. Both male and female healthy rabbits, weighing 1 kg were included. An acclimatization period of one week with twelve hours day and night cycles was provided to the rabbits. Adequate diet and water for drinking was provided. Animals were divided into three groups consisting of six rabbits in each with equal male to female ratio. Groups were named A, B, & C.

The amphotericin B preparation of Bristol-Myers Squibb, Fungizon, commercialized for clinical use was selected for the current study. It is a deoxycholate-complexed formulation of amphotericin B. Acute nephrtotoxic dose of 4mg/kg body weight was diluted in 5% Dextrose water in accordance with the instructions given by the manufactrur of Fungizone. It was diluted in ratio of 10ml Dextrose water/mg of drug. Intravenous route of administration was employed and a single acute nephrotoxic dose of 4mg/kg of body weight diluted in 40 ml of 5% dextrose water was infused very slowly in about 3-4 hours. Pediatric chamber connected to a butterfly needle was used for infusion in

Table-1: Comparison of results of renal functions of group A, B & C on day 1 & day 6: serum analysis:

		Day-1			Day-6			
Test	G-a	G-b		G-c	G-a	G-b		G-c
S. Bun (mmol/l)	4.96	6.43		5.94	6.11	21.18		8.28
	±0.25	±0.84		±0.11	±0.64	±2.39		±1.26
					p=<0.12*	*p*=<0.01		*p*=0.02*
S.Creatinine (µmol/l)	98.83	96.83		88.83	91.16	283		101.66
	±1.81	±5.92		±6.79	±4.01	±40.77		±4.81
					p= 09*	*p*=<0.01		*p*=0.02*
S.Sodium (mmol/l)	138.83	139.66		138.33	139.16	137.5		137.83
	±0.3	±0.8		±0.71	±0.47	±1.07		±0.53
					p=<61*	*p*=0.25*		*p*=0.17*
S.Potassium (mmol/l)	4.91	4.56		4.1	4.93	4.68		4.61
	±0.04	±0.11		±0.13	±0.04	±0.74		±0.3
					p=0.83*	*p*=0.38*		*p*=0.33*

Results represent as mean ± SEM (standard error of mean).
* Not Significant

marginal ear veins of rabbits. 0.9% Saline was used for sodium loading.

Group A (Control group) was injected 0.9% normal saline, 10 ml/kg/day10 intraperitoneal 11 for 5 days. Animals were sacrificed on 6th day, blood samples were collected and kidneys were preserved in 10% formaline for histopathology.

Group B received distilled water intraperitonially for first 4 days, followed by

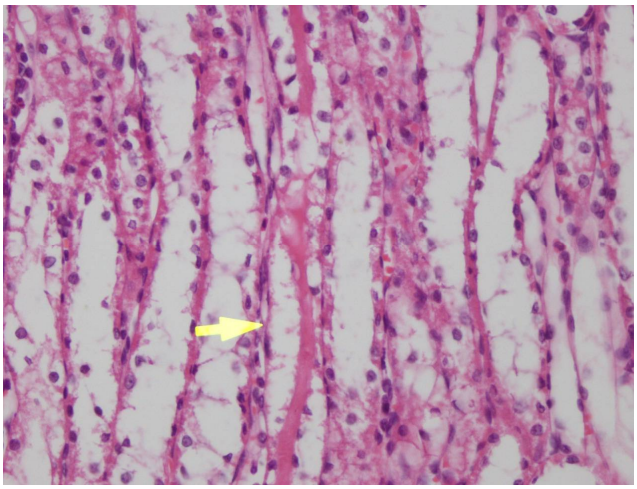

Figure-1: Microscopic structures of rabbit renal cortex of a rabbit from group B showing acute tubular necrosis (marked nephrotoxicity) in renal tubules. (X400).

single acute nephrotoxic dose of amphotericin B deoxycholate (Fungizone), 4 mg/kg 12 as slow intravenous infusion on 5th day. Animals were sacrificed 24 hours after infusion on 6th day, blood samples were collected and kidneys were preserved in 10% formaline for histopathology.

Group C was given 0.9% normal saline, 10 ml/kg, intraperitoneal for five days prior to single acute nephrotoxic dose of amphotericin B deoxycholate (Fungizone), 4 mg/kg as slow intravenous infusion on 5th day. Animals were sacrificed 24 hours after infusion on 6th day, blood samples collected and kidneys were preserved in 10% formaline for histopathology.

Collection of samples

1 ml blood was taken from marginal ear vein of each rabbits twice during the study, first sample as baseline on 1st day and second sample just before sacrificing the animal. Blood urea, serum creatinine, serum electrolytes (sodium and potassium) were checked.

RESULTS

The results of serum analysis are expressed as means + standard deviation calculated on computer using Independent T-test Test to find the difference within the group using statistical

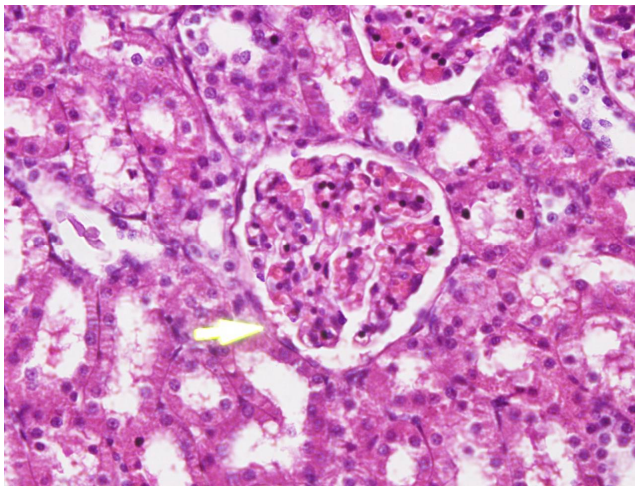

Figure-2: Microscopic structures of renal cortex of a rabbit from group C showing mesangealproliferation and loss of nuclei (mild nephrotoxicity) in some tubules (X400).

package for social science (SPSS) 21. In order to find the significance of the difference between two observations, “paired student’s t test” was applied. The difference between two observations is considered significant if the *p* value is less than 0.05. Microscopic examination was carried out on sections of rabbit kidney. Specimens were examined for tubular necrosis which was graded as follows[14]:

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.

Group A: The values of blood urea, serum creatinine, sodium and potassium on 6th day sample were almost similar to those on 1st day, *p*-value of all the parameters was not significant (table-1). Histology showed intact renal architecture with normal renal parenchyma and intact nuclei. There was no sign of necrosis and renal cortex was normal.

Group B: Blood urea and creatinine increased significantly, *p*-value of all the specimens was significant, being < 0.01. Changes in serum sodium and serum potassium were unremarkable, *p* value was not significant (Table-1). Histopathology showed grade-3 (marked) necrosis with extensive loss of nuclei (fig-1).

Group C: Rise in blood urea and creatinine was mitigated. Changes in serum sodium and serum potassium were unremarkable and *p*-value was not significant (table-1). Histopathology showed reduced, grade-1 (mild) necrosis with proliferation of mesangeal cells and loss of nuclei (fig-2).

DISCUSSION

Amphotericin is highly nephrotoxic drug. In one study chronic renal failure was observed in 44% of patients receiving more than a total of 4g of amphotericin B[15]. Amphotericin B has high affinity for biological membranes, resulting in binding to sterols, which is most likely responsible for its excellent antifungal properties, same activity may alter cellular membrane functions resulting in organ dysfunction[16].

Present work was based on the hypothesis that sodium loading can attenuate amphotericin B induced nephrotoxicity. This produced nephrotoxicity in rabbits from group B and C whereas group A acted as a control. Sharp rise in blood urea and serum creatinine along with grade-3 necrosis on histopathological analysis indicated severe nephrotoxicity in group B (table-1).

These findings are consistent with the results recorded in other studies[17,18]. However serum electrolytes in our work did not show any rise (table-1). The possible reason for this could be short interval of time (24hours) between the single acute nephrotoxic dose of amphotericin B and collection of blood samples for electrolyte estimation[18]. Group C showed marked mitigation of nephrotoxicity with saline loading as evident from normal levels of blood urea and serum creatinine (table-1) and a corresponding reduction in renal injury on histopathology (grade-I, mild necrosis). These results in Group C confirmed the protective role of sodium loading against amphotericin B induced nephrotoxicity.

Amphotericin B produces renal injury by a variety of mechanisms. Early in therapy there is a significant rise in creatinine. This is secondary to renal vasoconstriction of the afferent arteriole. The deoxycholate moiety is nephrotoxic and accounts for the differential renal toxicity of Amphotericin B deoxycholate as compared with lipid compounds. Additional tubular injury produces hypokalemia and hypomagnesemia and, probably less clinically significant, bicarbonate and amino acid loss. Work in dogs has suggested that Amphotericin B nephrotoxicity is caused by enhanced tubulo-glomerular feedback. Tubulo-glomerular feedback is a normal intra-renal mechanism whereby increased solute delivery to distal tubule results in afferent arteriolar vasoconstriction. Amphotericin B increases monovalent ion delivery to the distal tubule causing afferent arteriolar vasoconstriction, most likely due to local adenosine release. Other mechanisms of Amphotericin B induced nephrotoxicity suggested in the literature are direct toxic effects to the afferent arterioles and tubules and direct renal and systemic vasoconstriction. Within minutes after the intravenous injection of Amphotericin B is begun, renal blood flow is reduced and the production of urine is decreased, despite the maintenance of systemic blood pressure[19].

CONCLUSION

Present work shows that cost effective and simple method of sodium loading can be safely

practiced in our setting where liposomal formulations of amphotericin B are not used because of non-availability due to extremely high cost of therapy which is out of reach for majority of patients. As a result almost all patients receiving conventional amphotericin B therapy in our country are exposed to the risk of its severe nephrotoxicity in all setups like renal transplant, oncology and bone marrow stem cell transplant centers. Renal damage results in longer hospital stay, added cost of treatment and higher morbidity as well as mortality.

RECOMMENDATION

It is recommended that clinical studies with sodium loading with 0.9% saline may be carried out on indoor patients receiving amphotericin B for confirming the role of this inexpensive and least hazardous intervention in amelioration of amphotericin B induced renal damage.

CONFLICT OF INTEREST

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

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