DRUG INDUCED ACUTE KIDNEY INJURY: AN EXPERIMENTAL ANIMAL STUDY

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ABSTRACT

Objective: To assess the extent of drug induced nephrotoxicity in laboratory animals for determining the role and extent of iatrogenic kidney damage in patients exposed to nephrotoxic drugs in various clinical setups.
Study Design: Randomized control trail.
Place and Duration of study: Pharmacology department and animal house of Army Medical College from Jan 2011 to Aug 2011.
Material and Methods: Thirty six mixed breed rabbits were used in this study. Animals were randomly divided into six groups consisting of six rabbits in each. Groups were named A, B, C, D, E and F. Group A was control group. Group B was given 0.9% normal saline. Group C rabbits were given acute nephrotoxic single dose of amphotericin B deoxycholate. Group D received 0.9% normal saline 10ml/kg followed by amphotericin B infusion. Group E was injected acute nephrotoxic regimen of cyclosporine and amphotericin B infusion. Group F received saline loading along with acute nephrotoxic regimen of cyclosporine and amphotericin B infusion.
Results: Biochemical and histopathological analysis showed significant kidney injury in rabbits exposed to acute nephrotoxic doses of amphotericin B and cyclosporine. Toxicity was additive when the two drugs were administered simultaneously. Group of rabbits with saline loading had significantly lesser kidney damage.
Conclusion: Iatrogenic acute kidney damage is a major cause of morbidity in experimental animals exposed to such nephrotoxic drugs like amphotericin B and cyclosporine, used either alone or in combination. Clinical studies are recommended to assess the extent of iatrogenic renal damage in patients and its economic burden. Efficient and cost effective protective measure may be adopted in clinical setups against such adverse effects.

Keywords: Iatrogenic, Interstitial cells, Morbidity.

INTRODUCTION

Acute kidney injury (AKI) is a syndrome characterized by a rapid (hours to days) deterioration of kidney function[1]. Nephrotoxicity is frequently seen with many medications in common use today. This is not surprising given the renal bed's significant exposure to administered medication as it receives 25% of the resting cardiac output over a large endothelial surface area. With a high metabolic rate involving numerous enzymes and transtubular cell transport processes, renal glomerular, tubular and interstitial cells may come in contact with drugs and their metabolites at greater concentrations than expected. Some medications can affect the vasoregulatory mechanisms of the kidney to cause a functional limitation. Nephropathy is often recognized only when filtration is affected as seen by a rise in blood urea and serum creatinine, because there are as yet no good serologic markers to determine the subtle insults to the kidneys. In a 1 year survey involving 55 centers, the Societe de Nephrologie identified 18.3% of acute renal failure (ARF) cases as drug-related. These cases were diagnosed as either acute tubular necrosis (ATN) or acute interstitial nephritis (AIN)[2]. A study conducted in a tertiary care unit in Spain revealed that half of the ARF episodes developed during hospitalization in medical and surgical departments were drug-related[3]. The short-term

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effects of AKI are acute deterioration in renal function and uraemia[4].

MATERIAL AND METHODS

Thirty six mixed breed rabbits (both male and female) were purchased from the local market. Rabbits were 7-12 months of age and weighed between 1 and 1.7 kg at the beginning of the study. They were kept at standard laboratory conditions in animal house of Army Medical College Rawalpindi from Jan 2011 to Aug 2011 and acclimatized for one week. They were provided tap water ad libitum for drinking purpose and all rabbits consumed same standard diet consisting of grass, carrots, turnips, peas and grain. Twelve hour light and dark cycle was maintained[5].

Animals were randomly divided into six groups consisting of six rabbits in each. Groups were named A, B, C, D, E and F. Group A was control group that was kept without intervention and sacrificed on 6th day. Group B was given 0.9% normal saline, 10ml/kg/ day for five days and sacrificed on 6th day. In Group C no intervention was done for first 4 days, on 5th day rabbits were given acute nephrotoxic single dose of amphotericin B deoxycholate[6] (inj Fungizone, Bristol Myers Squibb, Turkey), 4mg/kg as slow IV infusion diluted in 5% dextrose water and sacrificed on 6th day. Group D received 0.9% normal saline 10ml/kg followed by amphotericin B infusion, 4mg/kg[7]. Group E was injected acute nephrotoxic regimen of cyclosporine[8] (inj sandimmune, Novartis, Pakistan), in a dose 25mg/kg/d divided in two equal doses for five days. On 5th day amphotericin B infusion was also given. Group F received saline loading along with acute nephrotoxic regimen of cyclosporine, amphotericin B infusion given on 5th day.

Blood samples were collected twice from each animal during the study. First sample determined baseline values whereas 2nd sample was taken just before sacrificing the animal. Blood urea, serum creatinine and serum electrolytes (sodium and potassium) estimation was done. Measurement of blood urea and serum creatinine still remain among the most important means for assessing the degree of renal damage[9,10]. All animals were sacrificed at the end of experiment, both kidneys removed and preserved in 10% formaline for histopathology. Microscopic examination was carried out on sections of rabbit kidney. Specimens were examined for tubular necrosis which was graded

Table: Effects of coadministration of amphotericin B and cyclosporine on renal functions of rabbit; Group A (Control) vs Group E (Amphotericin B + cyclosporine): Serum analysis:

	Day-1		Day-6	
Test	G-A	G-E	G-A	G-E
Serum urea	4.96	6.31	6.11	17.1
(mmol/l)	± 0.25	± 0.2	± 0.64	± 1.89
		p<0.0		
S.Creatinine	98.83	70.16	91.16	141.5
(µmol/l)	± 1.81	± 1.01	± 4.01	± 7.78
		p<0.01		
S.Sodium	138.83	142.5	139.16	133.66
(mmol/l)	± 0.3	± 1.56	± 0.47	± 2.12
		p<0.01		
S.Potassium	4.91	4.28	4.93	6.58
(mmol/l)	± 0.04	± 0.16	± 0.04	± 0.44
		p<0.01		

Results represent as mean ± SEM (standard error of mean) n=6.

as follows[11]:, 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.

RESULTS

No biological or histological evidence of renal injury observed from examination of blood and kidney tissue of animals from group A that served as control group. Similarly normal biological values and intact renal architecture noted in group B. Post dose blood samples of Group C revealed acute rise in blood urea and serum creatinine levels in excess of 200%. Serum electrolytes remained unchanged because of short post dose period of 24 hours[10]. Histopathology showed marked, grade-3 necrosis of renal tubules that showed marked nephrotoxicity. Group D served as saline loading group, showed significant nephroprotective role of saline loading. In group E, coadministration of amphotericin B and cyclosporine resulted in marked nephrotoxicity as evident from acute rise in blood urea, serum creatinine, hyponatremia and hyperkalemia, and marked, grade-3 necrosis of renal tubules (fig-1 & 2). Group F was marked as group of sodium loading with coadministration of amphotericin B and cyclosporine and showed significant nephroprotective role of saline loading in this group (table).

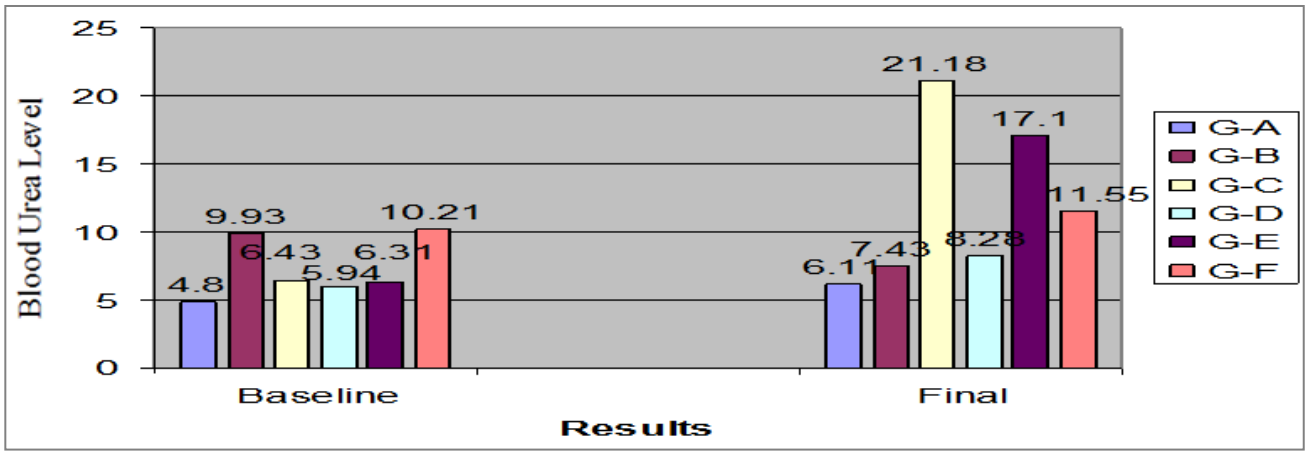

Figure-1: Blood urea levels before and after administration of drugs.

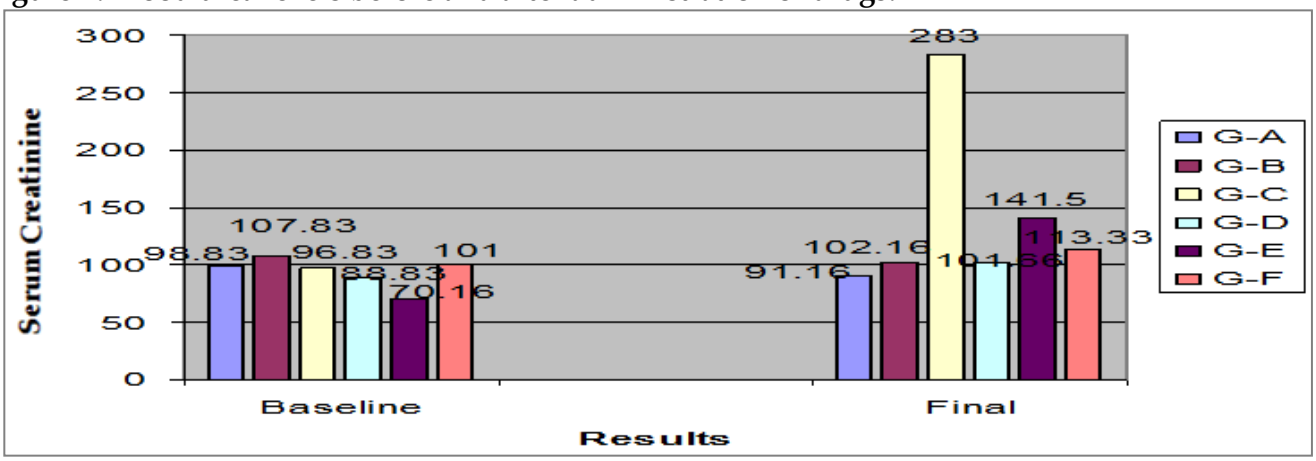

Figure-2: Serum creatinine levels before and after administration of drugs.

DISCUSSION

Amphotericin B is a polyene macrolide broad spectrum antibiotic with excellent anti-fungal properties[12]. Despite the development of a

number of new antifungal agents, amphotericin B formulated as a suspension remains one of the most effective broad-spectrum[13] antifungal agents available for clinical use in the treatment of systemic fungal infections[14] however some strains of *candida albicans* show resistance to it[15]. The drug must be administered intravenously and is associated with numerous side effects[16]. One of the major deterrents in its prescription is its nephrotoxicity[17]. Amphotericin B contains hydrophilic as well as lipophilic regions[18]. It binds to the sterol component of cell membrane and leads to alterations in cell permeability causing cell death. While amphotericin B has a higher affinity for the ergosterol component of the fungal cell membrane, it can also bind to the cholesterol component of the mammalian cell leading to cytotoxicity[19]. About 80% of patients administered with therapeutic doses of amphotericin B for three or more days develop laboratory evidence of renal injury[16]. In a study conducted in 2001, it was discovered that patients who received parenteral amphotericin B therapy and developed acute renal failure had substantially higher mortality, longer hospital stay, and higher cost of treatment than those who did not have acute renal failure[20]. Measurement of blood urea and serum creatinine still remains among the most important means for assessing the degree of renal damage[14].

Cyclosporine is a cyclic polypeptide with 11 amino acids of fungal origin. It was obtained from a fungus and introduced in 1977 as a highly selective immunosuppressant drug which has markedly increased the success of organ transplantations[21] and is widely used in transplant centres[22]. Cyclosporine has significant nephrotoxicity, it can cause abnormalities of glomerular filtration rate, proximal and distal tubular function resulting in hyperkalemia, hyponatremia and acidosis[23,24]. Cyclosporine has to be given over an extended period. This prolonged therapy can result in cyclosporine-induced nephrotoxicity. Acute and chronic nephrotoxicity of this drug is a major drawback of current immunosuppressive regimens[25]. Usually it has to be given concurrently with amphotericin B. The combined administration of amphotericin B and cyclosporine is frequent in patients undergoing stem cell transplantation which results in cumulative effect causing additive nephrotoxicity due to both nephrotoxic agents[26,27].

CONCLUSION

Iatrogenic acute kidney damage is a major cause of morbidity in experimental animals exposed to such nephrotoxic drugs like amphotericin B and cyclosporine, used either alone or in combination. Clinical studies are recommended to assess the extent of iatrogenic renal damage in patients and its economic burden. Efficient and cost effective protective measure may be adopted in clinical setups against such adverse effects[7].

CONFLICT OF INTEREST

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

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