Nephrotoxicity Reduction by Fixed Dose Combination of Cephalosporins and Aminoglycosides in *Mus musculus* Mice

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**Abstract:** Free radicals are causative factors for aminoglycoside induced renal toxicity. The aim of present study was to evaluate effect of fixed dose combination of cefepime+amikacin (Potentax) as well as ceftazidime+tobramycin (Tobracef) antibiotics on antioxidant enzymes (Superoxide dismutase, Catalase and Glutathione reductase) along with (free radical mediated damage) malonaldehyde levels and extracellular antioxidant enzymes (creatinine, total bilirubin and uric acid enzymes) in kidney tissue of *Mus musculus* mice. Present findings showed that the activities of the antioxidant enzymes were significantly lowered along with increase in MDA (malonaldehyde) levels and extracellular antioxidants after single treatment of aminoglycosides (amikacin and tobramycin) as compared to control group. A significant improvement in antioxidant enzymes along with significant decrease in creatinine, total bilirubin, uric acid and malonaldehyde (MDA) levels were observed in fixed dose combination of cefepime plus amikacin as well as ceftazidime+tobramycin treated groups compared to amikacin and tobramycin alone treated group. These results indicate that a fixed dose combination of cephalosporins with aminoglycosides using chemical vector mediated technology acts as an antioxidant and prevents nephrotoxicity induced by aminoglycosides.

**Key words:** Free radical, antibiotics, antioxidant enzymes, amikacin, nephrotoxicity

**INTRODUCTION**

Aminoglycosides (amikacin and tobramycin) are most important drugs in clinical use and also essential for the treatment of severe infections caused by gram-negative bacteria. These antibiotics are reported to cause nephrotoxicity, ototoxicity and neuromuscular blocks (Gilbert, 2000; Selimoglu, 2007; Oliveira et al., 2006). Neuromuscular blocks are rare, ototoxicity ranges from 0-62% and nephrotoxicity varies from 0-19% (Gilbert, 1995). The binding of aminoglycosides in vivo as well as in vitro with negatively charged membrane is associated with impairment of phospholipid catabolism, change in membrane permeability and membrane aggregation. Aminoglycosides bind to membranes, where they are endocytosed and accumulate with phospholipid within the tubular cell lysosomes. Due to increase accumulation of aminoglycosides results in a stepwise alteration of cell function and ultimately causes cell necrosis (Tan et al., 2003). The adverse effect of aminoglycosides has been attributed to the development of an array of alterations in proximal tubule epithelium followed by its destruction, thereby causing kidney dysfunction (Mingeot-Leclercq et al., 1999). Aminoglycosides administration is also reported to induce apoptosis (Lang and Liu, 1997), free radical generation (Klemens et al., 2003) and glomerular basement membrane alterations (Gilbert, 2000). Free radicals also play an important role in drug-induced damage to the kidney failure and other organs (Mingeot-Leclercq and Tulkens, 1999).
Cefapime and ceftazidime are cephalosporin class of antibiotics having potential of free radical scavenging properties (Cantin and Woods, 1993). A combination of cephalosporins plus aminoglycosides has been used for many years as empirical therapy because of broad spectrum of activity against pathogens. There are several studies which suggest that cephalosporins in combination with aminoglycosides prevent aminoglycosides induced toxicities (Yazaki et al., 2002). Chemical vector mediated technology was used to form synergistic combination of aminoglycosides and cephalosporins with antioxidant potential.

The purpose of the present study was to evaluate the effect of single administration of aminoglycosides and their fixed dose combination with cephalosporins on renal antioxidant enzymes, extracellular enzymes and malonaldehyde (MDA) levels in mice by using chemical mediated technology.

**MATERIALS AND METHODS**

**Chemicals**

All of the chemicals used in the present study were procured from Sigma, St. Louis, MO, USA. Other chemicals purchased locally, were of analytical grade. All of the antibiotics such as amikacin, tobramycin, cefepime plus amikacin (Potentox) and ceftazidime plus tobramycin (Tobracef) were obtained from Venus Remedies Ltd. India. The ratio of fixed dose combination of cefepime+amikacin and ceftazidime+tobramycin were 4:1 and 11:1, respectively. This study was conducted at Venus Medicine Research Centre, Baddi, HP.

**Animals and Treatments**

Thirty Mus musculus mice (weighing 30±5 g) were used in the experiment. The mice were fed standard pelleted diet and sterile water *ad libitum*. The mice were divided into five groups of six mice each. The respective drugs were intramuscularly administered every day for 7 days.

- Isotonic saline treated group
- Amikacin sulphate treated group (285×10⁻⁷ g g⁻¹ b.wt. day⁻¹)
- Tobramycin sulphate treated group (4×10⁻⁷ g g⁻¹ b.wt. day⁻¹)
- Ceftazidime plus Tobramycin treated group (341×10⁻⁷ g g⁻¹ b.wt. day⁻¹)
- Cefepime plus Amikacin fixed dose combination treated group (357×10⁻⁷ g g⁻¹ b.wt. day⁻¹)

Overnight fasted animals were sacrificed on 8th day and kidney tissue was taken out after perfusion. Kidney homogenates (15% w/v) were prepared in phosphate buffer-KCl solution containing 0.15 mol L⁻¹ KCl in 0.05 mol L⁻¹ NaH₂PO₄-NaHPO₄ buffer, pH 6.8. The enzyme preparation were left for at least 1 h at 0-40°C. All of the enzyme assays were carried out at 25°C.

**Enzyme Assays**

Superoxide dismutase (SOD) activity was determined using the method by Misra and Fridovich (1972). The reaction mixture consisted of 1.0 mL carbonate buffer (0.2 M, pH 10.2), 0.8 mL KCl (0.015 M), 0.1 mL of homogenate and distilled water to make the final volume to 3.0 mL. The reaction was started by adding 0.2 mL of epinephrine (0.025 M). The change in absorbance was recorded at 480 nm at 1 sec intervals for 1 min at 25°C. Suitable control, lacking the enzyme preparation was run simultaneously. One unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto oxidation of epinephrine.

Catalase activity was analyzed using the method by Luck (1965). The reaction mixture consisted of 0.3 mL phosphate buffer, (0.2 M, pH 6.8), 0.1 mL H₂O₂ (1 M) and distilled water to make the final
volume to 3.0 mL. The reaction was started by adding the suitable aliquot of homogenate preparation in enzyme linearity range. The change in the absorbance was recorded at 15 sec interval for one minute at 240 nm at 25°C. Suitable control was run simultaneously. One Unit of enzyme activity has been defined as the amount of enzyme that liberates half of the peroxide oxygen from H_2O_2 in 100 sec at 25°C.

Glutathione Reductase (GR) activity was measured using the method by Carlberg and Mannervik (1985). The reaction mixture consisted of 1.5 mL of potassium phosphate buffer (0.2 M, pH 7.0) containing 2 mM EDTA, 0.15 mL of 2 mM NADPH, 0.2 mL of 20 mM oxidised glutathione and added distilled water to make up the final volume to 3.0 mL. The reaction was started by adding the 0.1 mL of homogenate in the enzyme linearity range. The absorbance was measured at 340 nm for one minute at 15 sec intervals. Control lacking the enzyme was run simultaneously. One unit of GR activity is expressed as the amount of NADPH formed in one minute by 1 mL of enzyme preparation. Calculation of the enzyme activity was done by using the molar extinction coefficient of NADPH as 6.22 x 10^3.

Creatinine, uric acid and total bilirubin levels were determined by using commercially available diagnostic kits (Bayer Diagnostics India Ltd., Baroda, Gujarat, India).

Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation with respect of the malondialdehyde (MDA) formed, essentially according to Ohkawa et al. (1979). It was determined by thio barbituric reaction. The reaction mixture consisted of 100 µL of homogenate, 0.20 mL of 8.1% Sodium Dodecyl Sulphate (SDS), 1.5 mL of 20% acetic acid, 1.5 mL of 0.8% Thio Barbituric Acid (TBA) and distilled water to make up the volume to 4.0 mL. The tubes were boiled in water bath at 95°C for one hour, immediately cooled thereafter under running tap water and 1.0 mL of water and 5.0 mL of mixture of n-butanol and pyridine (15:1 v/v) was added and vortexed. The tubes were centrifuged at 3500 x g for 30 min. The upper layer was aspirated out and absorbance measured at 532 nm. The reference used was 1, 1, 3, 3 tetra ethoxy propane.

**Statistical Analysis**

The data obtained was analyzed statistically. All values are expressed as Mean±SD. One-way Analysis of Variance (ANOVA) with student-Newman-Keuls comparison test was used to determine statistical difference between the control and experimental groups and p<0.05 were considered statistically significant.

**RESULTS AND DISCUSSION**

The results in this study show a significant (p<0.001) decrease in the superoxide dismutase (SOD) activity in the amikacin and tobramycin treated groups as compared to the control group. The activity of SOD was significantly increased in the cefepime+amikacin treated group (p<0.05) as compared to the amikacin alone treated group. In the case of cefazidime+tobramycin treated group, the activity was increased but did not alter significantly as compared to tobramycin alone treated group. Both of the fixed dose combination of drugs were statistically significant (p<0.01) when compared with the control group (Fig. 1).

The catalase activity was found to be significantly decreased in the amikacin and tobramycin alone treated groups as compared to the control group (p<0.001 and p<0.01, respectively). In the case of fixed dose combination of cefepime+amikacin treated group, the catalase activity was increased significantly (p<0.05) as compared to the amikacin alone treated group. The combined treatment of cefazidime+tobramycin however did not show significant change in catalase activity in comparison to tobramycin alone treated group. In the case of combination therapy of cefepime + amikacin and cefazidime+tobramycin treated groups, the catalase activities were found to increase significantly (p<0.001 and p<0.01, respectively) and decrease compared to the control group (Fig. 2).
Fig. 1: Effect of aminoglycosides and its combination with cephalosprins on the SOD activity in kidney tissue. Values are expressed in Mean±SD. A: Amikacin treated group, C+\(A\): Cefepime plus Amikacin treated group, T: Tobramycin treated group and C+T: Ceftazidime plus Tobramycin treated group. **\(p<0.001\) highly significant, \(\ast p<0.01\) significant, \(\ast\ast p<0.05\) significant \(p>0.05\), ns: Not significant.

Fig. 2: Effect of aminoglycosides and its combination with cephalosprins on the catalase activity in kidney tissue. Values are expressed in Mean±SD. A= amikacin treated group, C+\(A\): Cefepime plus Amikacin treated group, T: Tobramycin treated group and C+T: Ceftazidime plus Tobramycin treated group. **\(p<0.001\) highly significant, \(\ast\ast p<0.01\) significant, \(\ast\ast\ast p<0.05\) significant \(p>0.05\), ns: Not significant.

The Glutathione Reductase (GR) activity was found to decrease significantly \(p<0.001\) in the amikacin and tobramycin alone treated groups compared to the control group. In the fixed dose combination groups, the activities were significantly increased \(p<0.001\) when compared to the single administration of aminoglycosides (Fig. 3).

A significant increase in MDA levels were observed between the amikacin \(p<0.001\) and tobramycin \(p<0.01\) treated groups as compared to the control group. The fixed dose combination of cefepime+amikacin and ceftazidime+tobramycin resulted in a decrease in the MDA levels as compared to the amikacin and tobramycin alone treated groups (Fig. 4).
Fig. 3: Effect of aminoglycosides and its combination with cephalosporins on glutathione reductase activity in kidney. Values are expressed in Mean±SD. A: Amikacin treated group, C+A: Cefepime plus Amikacin treated group, T: Tobramycin treated group and C+T: Cefazidime plus Tobramycin treated group. ***p<0.001 highly significant, **p<0.01 significant, *p<0.05 significant p>0.05, ns: Not significant.

Fig. 4: Effect of aminoglycosides and its combination with cephalosporins on the MDA level in kidney. Values are expressed in Mean±SD. A: amikacin treated group, C+A: Cefepine plus Amikacin treated group, T: Tobramycin treated group and C+T: Cefazidime plus tobramycin treated group. ***p<0.001 highly significant, **p<0.01 significant, *p<0.05 significant p>0.05, ns: Not significant.

The single dose therapy of amikacin and tobramycin treated groups resulted in significantly increased levels of creatinine and bilirubin levels when compared to the control group. When a combination of cefepime +amikacin and cefazidime+tobramycin was administered, the levels of both creatinine and bilirubin decreased and reached almost to the levels of that of the control groups (Fig. 5, 6).

Uric acid level was found to be very significantly increased in the amikacin as well as in the cefepime+amikacin treated groups (p<0.001) as compared to the control and the amikacin alone treated
Fig. 5: Effect of aminoglycosides and its combination with cephalosporins on creatinine level in kidney. Values are expressed in Mean±SD. A: amikacin treated group, C+A: Cefepime plus Amikacin treated group, T: Tobramycin treated group and C+T: Cefazidime plus Tobramycin treated group. ***p<0.001 highly significant, **p<0.01 significant, *p<0.05 significant p>0.05, ns: not significant

Fig. 6: Effect of aminoglycosides and its combination with cephalosporins on total bilirubin level in kidney. Values are expressed in Mean±SD. A: amikacin treated group, C+A: Cefepime plus Amikacin treated group, T: Tobramycin treated group and C+T: Cefazidime plus Tobramycin treated group. p<0.001 highly significant, p<0.01 significant, p<0.05 significant p>0.05, ns: not significant

group, respectively. In the case of tobramycin alone and cefetazidime+tobramycin treated group, the uric acid level was remained unchanged statistically as compared to the control and the tobramycin alone treated group (Fig. 7).

Free radicals and their derivatives are capable of causing extensive damage at cellular and tissue levels resulting in a variety of untoward patho-physiological conditions (Pandey et al., 2000). Aminoglycosides causes tissue injury in the form of nephrotoxicity and ototoxicity due to generation
of free radical. Several studies have reported that tobramycin and amikacin cause nephrotoxicity, ototoxicity and alterations in cochlear antioxidant enzyme activities (Klemens et al., 2003, Rybak and Somani, 1999).

The formation of the free radicals is also responsible for renal injury due to aminoglycosides. Aminoglycosides enhance excessive production of hydrogen peroxide by the renal cortex which inhibit the synthesis of phospholipases A<sub>2</sub> and glutathione in rats (Soejima et al., 2000). Due to the induction of aminoglycosides, the ratio of free radical generating and free radical scavenging enzymes may be disturbed which leads to disruption of signal transduction pathway and increases the cellular permeability by acting on membrane phospholipids. The binding of these aminoglycosides with cellular membrane causes impairment of phospholipid catabolism, changes in membrane aggregation (Van Bambeke et al., 1995) and reduces the activities of phospholipases (Kalogianides, 1992; Laurent et al., 1982).

Cephalosporins however are a class of antibiotics such as cefepime and ceftazidime that contain free radical scavenging potential. Cefepime has low in vitro affinity for the major chromosomally mediated lactamases and good stability against enzymatic hydrolysis (Tumah, 2005). There have been several reports suggesting that cephalosporins gives protection against HOCl-driven oxidative injury. This defense is a consequence of a direct drug scavenging capacity towards HOCl (Lapenna et al., 1995).

Various studies have been reported suggested that amikacin and tobramycin alter antioxidant defence mechanism (Yazar et al., 2003). Aminoglycosides are rich in primary amines and possess cytoprotective properties but would not be expected to protect extracellular sulphhydril group against free radical-mediated oxidation. Cephalosporins are thioether group containing antibiotics which are very effective in preventing the free radical-mediated oxidation of sulphhydril groups (Cantin and Woods, 1993). When aminoglycosides combined with the cephalosporins, exhibits more synergistic effect than single therapy and prevents against the severe infections.

The present finding demonstrates that single treatment of amikacin and tobramycin aminoglycosides significantly reduce the antioxidant enzymes activities (SOD, Catalase and
Glutathione reductase along with increased free radical mediated damage (as evidenced by enhanced MDA levels) as well as some extracellular antioxidants (Creatine, Uric acid and Total bilirubin) in treated mice. These biological parameters indicate that the administration of single antibiotic causes renal toxicity. Similar finding was reported with other aminoglycosides such as streptomycin and gentamycin in kidney and heart (Vijayalakshmy et al., 1992; Ozturk et al., 1997). Several studies have reported that the reduction of aminoglycoside nephrotoxicity by cephalosporin class of antibiotics is due to their intrinsic nephroprotective potential rather than the concentration of aminoglycosides in the kidney (Beauchamp et al., 1994).

Present findings demonstrate that the fixed dose combination of cefepime and amikacin and ceftazidime and tobramycin effectively protects against amikacin and tobramycin induced nephrotoxicity. These findings show that the levels of antioxidant enzymes and other biochemical parameters were improved along with significant decrease in the level of MDA. Present data support the concept that a fixed dose combination of the cephalosporin with aminoglycosides has antioxidant and free radical scavenging potential besides the antibacterial activity. It is postulated that the combination therapy of cefepime plus amikacin (Potentox) and ceftazidime plus tobramycin (Tobrace) scavenge off aminoglycoside induced free radical generation, oxidative stress and tissue injury. In addition to proven synergy of ceftazidime+tobramycin and cefepime+amikacin, antioxidant characteristics of these combinations will also scavenge oxidative stress induced by pathogens and thus will improve efficacy of the treatment.

CONCLUSION

The study concluded that using chemical vector mediated technology, a fixed dose combinations of cephalosporins and aminoglycosides possess antioxidant and free radical scavenging potential which contributes in improving the efficacy and safety profiles of these combinations.

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REFERENCES


