

Comparative Safety Evaluation of Potentox® Vs Co-Administration of Cefepime And Amikacin In Healthy Albino Rat

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Abstract

Context: Aminoglycosides and cephalosporins are widely used antibacterial agents for the treatment of both severe aerobic Gram negative and Gram positive bacterial infections. Though the combination therapy scores over single antibiotic therapy in terms of efficacy but the risk of associated nephro-toxicity and hepato-toxicity is a cause of concern.

Objective: The present study was undertaken to determine the comparative effect of co-administration of cefepime and amikacin one after the other versus Potentox® (single injection combination of cefepime and amikacin supplemented with chemical vector having antioxidant property) and their effect on liver and kidney functions in a healthy albino rat model. The purpose is to compare both regimens for comparative nephro and hepato-toxicity profile and effect of chemical vector, in Potentox®.

Materials and Methods: Eighteen healthy albino rat were used in the experiment and divided in three groups containing six each. The respective drugs (amikacin, cefepime, Potentox®) were administered through intravenous route for 10 days. At the end of 3rd and 10th days of treatments blood samples were collected and tests were performed for catalase activity, reduced glutathion, total thiol, malonaldehyde, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, creatinine, uric acid and urea.

Results: Experimental results showed that catalase activity, reduced glutathion and total thiol levels decreased significantly in cefepime followed by amikacin treated group, while no significant change occurred in Potentox® treated group. The malonaldehyde, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, creatinine, uric acid and urea levels were significantly increased in cefepime followed by amikacin treated group and this increase was not of much significance in Potentox® treated group.

Conclusion: These findings reveals that the presence of chemical vector in Potentox® has yielded a significant free radical scavenging property which may contribute in decreasing the aminoglycoside induced nephrotoxicity and hepatotoxicity.

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INTRODUCTION

Empirical use of broad-spectrum antibiotics in febrile neutropenic patients has been shown to significantly reduce the morbidity and mortality from severe infection, in particular gram-negative bacteria. As per Infectious Diseases Society of America (IDSA) guidelines, for the use of antimicrobial agents in neutropenic patients with fever, published in 1990,

Ceftazidime a third generation cephalosporin having strong antimicrobial activity against *Pseudomonas aeruginosa*, but limited activity against methicillin-susceptible *Staphylococcus aureus* and streptococci and Cefepime is a fourth-generation cephalosporin with activity against both methicillin-susceptible *S. aureus* and *P. aeruginosa*, have been approved as empirical monotherapy and followed till late 90s. In 2000 empirical use of broad-spectrum antibiotic combinations particularly of a beta-lactam antibiotic and an aminoglycoside has been recommended due to rapid emergence of gram negative resistant strains. The combination of an aminoglycoside and an antipseudomonal beta-lactam has been commonly used as empirical therapy for febrile neutropenia after these guidelines. In this respect, the 2002 IDSA guidelines (Hughes *et al.*, 2002) febrile neutropenia be categorized into 2 risk groups: low risk and high risk and it is necessary to specify predictive factors for selecting high-risk patients, to prevent kidney-function alterations associated with the addition of an aminoglycoside to a single-agent regimen.

The combination of aminoglycoside and antipseudomonal cephalosporin is also recommended for Nosocomial pneumonia. A published review article in 2010 analyzed the management of HAP (hospital-acquired pneumonia) and VAP (ventilator-associated pneumonia) based on the guidelines by the following organizations : American Thoracic Society and Infectious Diseases Society of America, Latin American Thoracic Society, South African Thoracic Society , Japanese Respiratory Society, Portuguese Society of Pulmonology and Portuguese Intensive Care Society, Sociedade Brasileira de Pneumologia, Association of Medical Microbiology and Infectious Diseases of Canada, and British Society for Antimicrobial Chemotherapy. All of the reviewed guidelines recommend combination therapy for patients at risk of infection with multi-drug resistant pathogens (Thomas, 2010).

Several authors have reported superior efficacy of combination therapy in high risk patients but the risk of aminoglycosides induced nephrotoxicity and hepatotoxicity at higher doses is always there. Though aminoglycosides antibiotics have long been used for treating severe, hospital-acquired infections despite their beneficial effects, aminoglycosides have considerable nephrotoxic and hepatotoxic side effects. Amikacin induced free oxygen radical generation plays an important role in drug induced damage to the liver, kidneys and other organs (Conlon *et al.*, 1999; Leclercq *et al.*, 1999) and leads to nephrotoxicity and ototoxicity (Rybak *et al.*, 2005). The toxicity of aminoglycosides has been widely studied (Klemens *et al.*, 2003). It has been reported that renal damage can in turn leads to liver injury due to aminoglycosides (Martines *et al.*, 1988). To minimize the toxicity caused by aminoglycosides, Venus Remedies Limited, developed a fixed dose combination of cefepime and amikacin supplemented with chemical vector (Potentox®) using chemical vector mediated technology.

Chemical vector mediated technology is used to provide compatibility of cephalosporins and aminoglycosides without interfering with the pharmacokinetic property of drug component and later prevents the free radical mediated oxidative damage. Keeping this in view, the present study was planned to determine the comparative effect on hepato and nephrotoxicity of Potentox® a new single unit combination in comparison to co-administration of cefepime hydrochloride (herein after referred to as cefepime) followed by amikacin sulphate (herein after referred to as amikacin) as individual therapies. Antioxidant property, effect of LFT(liver function tests) and RFT (renal function tests) were evaluated on the basis of different tests results including catalase activity, levels of reduced glutathion (GSH), malonaldehyde (MDA), total thiol (t-SH), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline

phosphatase (ALP), creatinine, uric acid and urea in albino rat model.

MATERIALS AND METHODS

Chemicals

All the biochemicals used in the present study were procured from Sigma, St. Louis, MO, USA. Other chemicals were purchased locally and they were of analytical grade. Potentox® was provided by Sponsor Venus Pharma GmbH, Germany where as Cefepime Hydrochloride for Injection (Maxipime, of Bristol-Myers Squibb) and Amikacin sulphate (Bristokacin of Bristol-Myers Squibb) were procured from market on behalf of sponsor for the study. The ratio of combination of cefepime plus amikacin in Potentox® was 4:1.

Animals and Treatments

Eighteen healthy albino rat (age 3.5 to 4.0 months; weighing 150-200 g) were used in the experiment. The rats were fed with standard pelleted diet and sterile water *ad libitum*. Later, the rats were divided into three groups containing six rats each as given below. The doses administered to rats are described below:

Control group (Isotonic saline treated group)

Potentox® treated group (232.5 mg/Kg body weight/ 12 hr)

Cefepime (206.64 mg/Kg body weight/12hr) followed by Amikacin (46.5 mg/Kg body weight/12hr) administered through separate injections, treated group.

The respective drugs were administered through intravenous route for 10 days. At the end of 3rd and 10th days of treatments, 1ml blood samples were drawn in heparinized vials from the heart by cardiac puncture under the light ether anesthesia. Separated Plasma samples were diluted 10 times with chilled distilled water, left for at least 1hr at 0-4°C before the estimation of enzyme assay and biochemical parameters.

Biochemical analysis

Estimation of Catalase

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

Estimation of GSH

The reduced glutathione was estimated by the method of Chandramohan *et al.* (2009) with slight modifications. Plasma preparation 0.5 ml was mixed with 0.5 ml of 5% (w/v) TCA reagent and kept for 10 min, proteins were precipitated and filtrate was removed vary carefully after centrifuge at 3500 rpm for 15 min. Take 0.25 ml of Na₂HPO₄ (4.25%) and 0.04 ml of DTNB (0.04%). A blank sample was prepared in a similar manner using distilled water in place of filtrate. The pale yellow colour was developed and OD was measured at 412 nm wavelength by spectrophotometer.

Estimation of Lipid Per oxidation

Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation in the term of malonaldehyde (MDA) formed, essentially according to Ohkawa *et al* (1979) . It was determined by thio barbituric reaction. The reaction mixture consisted of 100 µl of diluted plasma, 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 later water of volume 4.0 ml was added for final makeup. The tubes were boiled in water bath at 95°C for one hour and cooled immediately under running tap water. Added 1.0 ml of water and 5.0 ml of mixture of n-butanol and pyridine (15:1 v/v) and vortexed. The tubes were centrifuged at 3500 rpm for 30 minutes. The upper layer was aspirated out and optical density was measured at 532nm. The

reference standard used was 1,1,3,3 tetra ethoxy propane.

Estimation of total thiol

Total sulphahydril content in diluted blood was analysed by the method of Hu (1994). A diluted plasma (0.2ml) was taken in test tubes and added 0.6 ml of Tris EDTA buffer (Tris 0.25M, EDTA 20mM; pH 8.2) followed by addition of 40 µl of 10 mM of dithiobis nitrobenzoic acid (DTNB in methanol) and make the total reaction upto 4.0 ml by adding 3.16 ml of methanol and all test were sealed and colour was developed for 15-20 min, followed by centrifugation at 3000g for 10-15 min at room temperature, the absorbance of the supernatant was measured at 412 nm.

Serum levels of SGOT, SGPT, ALP, Creatinine, Uric acid and Urea were estimated on Erba Smart Lab (Transasia Biomedicals Ltd, Mumbai India) biochemistry analyzer using diagnostic kits (Transasia Biomedicals Ltd, Mumbai India) as per GLP.

STATISTICAL ANALYSIS

All values are expressed in mean ± SD. One-way analysis of variance (ANOVA) with student-Newman-Keuls comparison test was done to determine the statistical difference between control and experimental groups. P values <0.05 were considered statistically significant.

RESULTS

Table-1 presents the plasma levels of catalase, GSH, MDA, total thiol, SGOT, SGPT, ALP, creatinine, uric acid and urea in control, cefepime followed by amikacin and Potentox® treated groups.

Catalase activity decreased significantly ($p < 0.001$) in cefepime followed by amikacin treated group as compared to control group on 3rd and 10th days of treatments. While non significant changes ($p > 0.05$) in the catalase activity were observed in Potentox® treated group as compared to control group on 3rd and 10th days of treatments.

GSH levels were significantly decreased ($p < 0.01$ and $p < 0.001$) in cefepime followed by amikacin treated group as compared to control group on 3rd and 10th days of treatments, respectively. No significant change ($p > 0.05$) in the levels of glutathione were observed in Potentox® treated group as compared to control group on 3rd and 10th days of treatments, respectively.

A significant increase ($p < 0.01$, $p < 0.001$) in MDA levels were observed in cefepime followed by amikacin treated groups as compared to control group on 3rd and 10th days of treatments, respectively. No significant changes ($P > 0.05$) were observed in MDA levels as compared to control group in Potentox® treated group on 3rd day of treatments, while a significant increase ($p < 0.05$) in MDA levels were observed in Potentox® treated group on 10th day of treatments as compared to control group.

There is significant decrease ($p < 0.001$) in levels of total thiol in cefepime followed by amikacin treated group as compared to control group on 3rd and 10th days of treatments. No significant changes ($p > 0.05$) were observed in t-SH levels in Potentox® treated group as compared to control group on 3rd and 10th days of treatments.

A significant increase ($p < 0.01$, $p < 0.001$) in SGOT levels were observed in cefepime followed by amikacin treated group as compared to control group on 3rd and 10th days of treatments, respectively. SGOT levels showed a non significant ($p > 0.05$) change in Potentox® treated group as compared to control group on 3rd and 10th days of treatments, respectively.

Blood levels of SGPT increased significantly ($p < 0.01$, $p < 0.001$) in cefepime followed by amikacin treated group as compared to control group on 3rd and 10th days of treatments. No significant change ($p > 0.05$) were observed in levels of SGPT in Potentox® treated group as compared to control group on 3rd and 10th days of treatments.

ALP level increased significantly ($p < 0.001$) in cefepime followed by amikacin treated group as compared to control group on 3rd and 10th days of treatments. A non significant change ($p > 0.05$) in level of ALP was observed in Potentox® treated group on 3rd day of treatments as compared to control, while Potentox® caused a significant increase ($p > 0.05$) in level of ALP on 10th day of treatments as compared to control group.

Cefepime followed by amikacin caused a significant increase ($p < 0.01$, $p < 0.001$) in blood creatinine level as compared to control group on 3rd and 10th days of treatments, respectively. The change in blood creatinine level was not significant ($p > 0.05$) in Potentox® treated group as compared to control group on 3rd day of treatments, while a significant increase ($p > 0.05$) in the creatinine level was observed in Potentox® treated group as compared to control group on 10th day of treatments.

Uric acid level increased significantly ($p < 0.001$) in cefepime followed by amikacin treated group as compared to control group on 3rd and 10th days of treatments. A non significant ($p > 0.05$) change in the levels of uric acid was observed in Potentox® treated group as compared to control group on 3rd day of treatments, while a significant ($p < 0.05$) increase in uric acid level was observed in Potentox® treated group as compared to control group on 10th day of treatments.

Blood urea level increased significantly ($p < 0.001$) in cefepime followed by amikacin treated group as compared to control group on 3rd and 10th days of treatments, respectively. A non significant change ($p > 0.05$) in levels of blood urea were observed in Potentox® treated group as compared to control group on 3rd day of treatments, while a significant increase ($p < 0.05$) in the levels of urea were observed in Potentox® treated group as compared to control group on 10th day of treatments.

DISCUSSION

Cefepime is a cephalosporins class antibiotic that has free radical scavenging potential. It has low *in-vitro* affinity for major chromosomally mediated lactamases and good stability against enzymatic hydrolysis (Tumah, 2005). The free radical scavenging properties of cefepime exhibit synergistic effect on antimicrobial property of amikacin and prevent the depletion of antioxidant levels as compared to single treatment of antibiotic.

Aminoglycosides are one of the common drugs which may induce oxidative stress by forming drug-derived radicals that not only deplete the antioxidant defenses but also react directly with biomolecules. Aminoglycosides disrupt the signal transduction pathway and increases the cellular permeability by acting on membrane phospholipids and have the usual adverse effects of ototoxicity, renal toxicity, neuromuscular block, allergic reactions (Laurent *et al.*, 1990; Schacht *et al.*, 1986). Their use is limited in the clinical practices due to side effect of ototoxicity and nephrotoxicity (González *et al.*, 1978; Carreer *et al.*, 1998; Yazaki *et al.*, 2002).

Amikacin has been reported to alter activities of antioxidant enzymes such as Catalase, Glutathione peroxidase (GSH-px), Glutathione-S-transferase (GST) and Glutathione reductase (GR) in various tissues (Bellés *et al.*, 2007). The reduced enzyme activity in the amikacin group is because of impaired function of the anti-oxidant pathway (Klemens *et al.*, 2003). Bendush *et al.*, (1976) reported that SGOT level increases in patients receiving aminoglycoside injection. It has been postulated that aminoglycoside induced free radical generation and alteration in antioxidant enzyme activities may be one of the cause of tissue injury. Amikacin alters liver glycogen phosphorylase activity and leads to decrease the liver glycogen content (Lietz *et al.*, 1990).

Experimental result reveals that the activity of antioxidant enzyme, catalase, decreased significantly in cefepime followed by amikacin

treated group which are in accordance with observations made by different authors earlier, while no significant change observed in Potentox® treated group as compared to control. Also the levels of GSH showed no significant change in Potentox® treated group, while a significant decrease occurred in cefepime followed by amikacin treated group, suggesting that cefepime in combination with amikacin reversed the adverse effects of amikacin on antioxidants level and in reversal activity of antioxidant enzymes to a certain extent due to presence of chemical vector CV001, suggesting that chemical vector (CV001) prevents the deterioration of anti-oxidant activity.

Potentox® also reversed the toxic effects of cefepime and amikacin in liver functions. The total thiol levels decreased significantly in cefepime followed by amikacin treated group, while no significant change occurred in Potentox® treated group. The free radical mediated damage MDA level, SGOT, SGPT, ALP levels were significantly increased in cefepime followed by amikacin treated group and this increase was not of much significance in Potentox® treated group.

Serum creatinine, uric acid and urea levels were measured to check drug induced nephrotoxicity. Blood levels of these were significantly increased in cefepime followed by amikacin treated group and this increase was not of much significance in Potentox® treated group, indicating the protective properties of chemical vector.

CONCLUSION

From the above results, it can be concluded that due to the chemical vector (CV001) mediated enhanced antioxidant property and free radical scavenging property of Potentox® (a novel combination of cefepime and amikacin supplemented with chemical vector), drug induced toxicity of amikacin (ototoxicity, hepatotoxicity and nephrotoxicity) could be reversed. Hence use of

Potentox as empirical therapy may be considered to be more safe in place of separate co-administration of individual antibiotics.

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Table-1: Summary of Biochemical Analysis

Parameters	Days	Control group	C+ A(Cefepime+ Amikacin) group	Potentox® group
Catalase activity (mmole/min/ml)	3 rd	538.57 ± 20.58	443.99 ± 30.08 ^a	526.56 ± 17.62 ^d
	10 th	533.8 ± 30.31	351.23 ± 30.89 ^a	518.06 ± 27.96 ^d
GSH (mmole/ml)	3 rd	4.56 ± 0.62	3.5 ± 0.26 ^b	4.31 ± 0.47 ^d
	10 th	4.79 ± 0.54	2.11 ± 0.22 ^a	4.39 ± 0.41 ^d
MDA (µmole/ml)	3 rd	4.58 ± 1.03	7.2 ± 1.41 ^b	5.5 ± 1.2 ^d
	10 th	4.83 ± 0.27	8.5 ± 0.76 ^a	5.7 ± 0.5 ^c
t-SH (µmole/ml)	3 rd	531.23 ± 25.46	417.12 ± 14.25 ^a	504.98 ± 23.45 ^d
	10 th	545.23 ± 28.53	340.12 ± 18.47 ^a	515.12 ± 27.12 ^d
SGOT (mg/dL)	3 rd	55.31 ± 2.69	62.00 ± 2.06 ^b	57.0 ± 3.53 ^d
	10 th	57.31 ± 4.42	112.00 ± 1.4 ^a	60.2 ± 3.78 ^d
SGPT (mg/dL)	3 rd	58.2 ± 3.54	68.4 ± 5.54 ^b	62.3 ± 2.76 ^d
	10 th	59.3 ± 2.09	124.3 ± 3.53 ^a	64.3 ± 4.71 ^d
ALP (mg/dL)	3 rd	262.71 ± 15.42	305.00 ± 18.01 ^a	266.00 ± 5.02 ^d
	10 th	263.00 ± 16.09	410.00 ± 11.12 ^a	245.00 ± 8.45 ^c
Creatinine (mg/dL)	3 rd	0.41 ± 0.01	0.58 ± 0.14 ^b	0.45 ± 0.04 ^d
	10 th	0.47 ± 0.02	1.06 ± 0.13 ^a	0.65 ± 0.15 ^c
Uric acid (mg/dL)	3 rd	2.13 ± 0.1	2.95 ± 0.16 ^a	2.48 ± 0.5 ^d

	10 th	2.11 ± 0.06	3.49 ± 0.33 ^a	2.4 ± 0.13 ^c
Urea (mg/dL)	3 rd	34.91 ± 1.81	42.51 ± 3.99 ^a	37.02 ± 0.87 ^d
	10 th	35.4 ± 1.01	52.58 ± 1.51 ^a	37.00 ± 1.03 ^c

All values are Mean ± SD. Where C; Control group , C+A; Cefepime plus Amikacin treated group, Potentox[®] group. Newman Keul test performed between control group vs treated groups at different days treatment for statistical analysis.

a= Statistical significant $p < 0.001$

b= Statistical significant $p < 0.01$

c= Statistical significant $p < 0.05$

d= Non significant $p > 0.05$