IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
June 2017 Vol.:6, Issue:4
© All rights are reserved by Mathew George et al.

Free Radical Scavenging Activity of Fixed Dose Combinations – Cephalosporin and Aminoglycoside Analougs (VRP001 & VRP002)

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Mathew George*, Lincy Joseph, Vikas Chaudhary, Saira Susan Varghese

Pushpagiri College of Pharmacy, Medicity Campus, Thiruvalla.

Submission: 2 June 2017
Accepted: 7 June 2017
Published: 25 June 2017

HUMAN JOURNALS
www.ijsrm.humanjournals.com

Keywords: Free radical scavenging activity, aminoglycoside, cephalosporin, anti-oxidant

ABSTRACT

Fixed Dose Combinations (FDC) are the combination of two or more active drugs in a single dosage form. The idea of combining two or more drugs with complementary mode of actions is to produce activity of desired therapeutic effect but with reduced or no adverse effects. Antibiotic combination products represent incremental rather than radical change, both of which are important for the progress of pharmaceutical technology. Aminoglycosides in combination with cephalosporins synergistically kill various pathogens and broaden the bactericidal spectrum against gram positive and gram negative bactreria[1]. Cephalosporins are beta-lactam antibiotics that share mechanism of action and similar structure with penicillins and also possess free radical scavenging potential. Free radical scavenging activity leads to the reduction of oxidative stress[2].

INTRODUCTION

Antioxidants are an enzyme or another organic molecule that can counteract the damaging effects of oxygen in the tissues. An imbalance between oxidants and antioxidants leads to oxidative stress. The presence of oxidative stress can be investigated either by detection of reactive oxygen species (ROS) and damaged products of essential biomolecules, loss of individual antioxidants like ascorbate or glutathione, malnutrition, excessive production of hydrogen peroxide, activation of natural radical producing systems. Excessive oxidative stress produces DNA damage, rise in intracellular free calcium and iron, damage to proteins and lipid peroxidation and finally results in cell injury and death. Aminoglycosides in combination with cephalosporins synergistically kill various pathogens and broaden the bactericidal spectrum against gram positive and gram negative bactreria[1]. Cephalosporins are beta-lactam antibiotics that share mechanism of action and similar structure with penicillins and also possesses free radical scavenging potential. Free radical scavenging activity leads to the reduction of oxidative stress[2]. In the present work, the free radical scavenging activity of FDC of cephalosporins and aminoglycosides is determined by enzyme assay - Superoxide dismutase (SOD) assay, Catalase assay, Measurement of malondialdehyde (MDA) level, Estimation of creatinine and statistical analysis by ANOVA.

MATERIALS AND METHODS

Chemicals

VRP001 and VRP002 are fixed dose combination (FDC) drugs.

VRP001 is a FDC consist of A (Amikacin sulfate) and B (Cefepime plus)

VRP002 is a FDC that consist of C (Tobramycin sulfate) and D (Ceftazidime plus)

The ratio of VRP001 and VRP002 is 4:1.

The control groups were treated with isotonic saline.

A group treated with 28.5 mg/kg body weight/day

VRP001 group treated with 35.7 mg/kg body weight/day

C group treated with 04.0 mg/kg body weight/day

VRP002 group treated with 34.1 mg/kg body weight/day

The respective drugs were administered intramuscularly for 7 days. At the end of treatment, 1ml blood was drawn in heparinised vials from heart by cardiac puncture under the light ether anesthesia. Blood samples were diluted 10 times with chilled distilled water, left for at least 1 hour at 0-4^{0}C before the estimation of the enzyme assay.

Animals

All the experiments were carried out using the *Mus musculus* mice (age 3.5 to 4) and male Wistar rats (age 3 to 4 months) procured from Venus Remedies Ltd, Baddi, HP. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol and the care of laboratory animals was taken as per the guidelines of CPCSEA. The registration number that was approved for animals was VMRC/BC/1045/08.

Enzyme Assay

Superoxide dismutase (SOD) assay[3]

The reaction mixture consisted of 1 ml carbonate buffer (0.2M, PH 10.2), 0.8 ml KCl (0.015M), 0.1 ml of blood and water to make the final volume to 3 ml. The reaction was started by adding 0.2 ml of epinephrine (0.025M). The change in the absorbance was recorded at 480 nm at 15 second interval for one minute at 25^{0}C.Suitable control lacking enzyme was run simultaneously. One unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of autooxidation of epinephrine.

Catalase assay

The reaction mixture consisted of 0.3 ml phosphate buffer (0.2M, pH 6.8), 0.1 ml H_2O_2 (1M), and water to make the final volume to 3 ml. The reaction was started by adding the suitable aliquot of enzyme preparation. The change in the absorbance was recorded at 240 nm at 15 second interval for one minute at 25^{0}C.Suitable control was run simultaneously. One unit of enzyme activity is defined as the amount of enzyme that liberates half of the peroxide oxygen from H $_2O_2$ in 100 seconds at 25^{0}C.

Measurement of Malondialdehyde (MDA) level

Free radical mediated damage was assessed by measurement of the extent of lipid peroxidation in terms of malondialdehyde MDA formed. It was determined by thiobarbituric reaction. The reaction mixture consisted of 100ml of diluted blood, 0.20ml of 8.1% sodium dodecyl sulfate, 1.5ml of 20% acetic acid, 1.5ml of 0.85 thiobarbituric acid and water to make up the volume to 4ml. The tubes were boiled in water bath at 95^0C for 1 hour and cooled immediately under running tap water. 1ml of water was added to 5ml of mixture of n-butanol and pyridine (15:1) and vortexed. The tubes were centrifuged at 3500rpm for 30 minutes. The upper layer as aspirated out and optical density was measured at 532nm. The reference standard used was 1,1,3,3-tetra ethoxy propane.

Estimation of creatinine

Creatinine level was determined by the alkaline picrate method using diagnostic kits.

RESULTS AND DISCUSSION

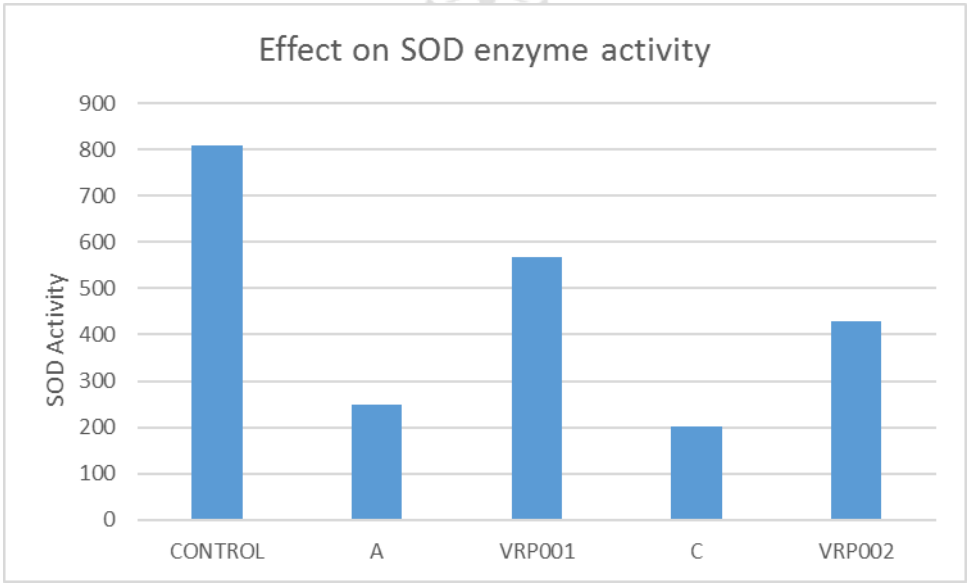

Figure 1: Effect on SOD antioxidant enzyme activity

The administration of aminoglycosides A and C significantly decreased SOD antioxidant enzyme activity, suggesting that the aminoglycosides induced oxidative stress could be cause of nephrotoxicity

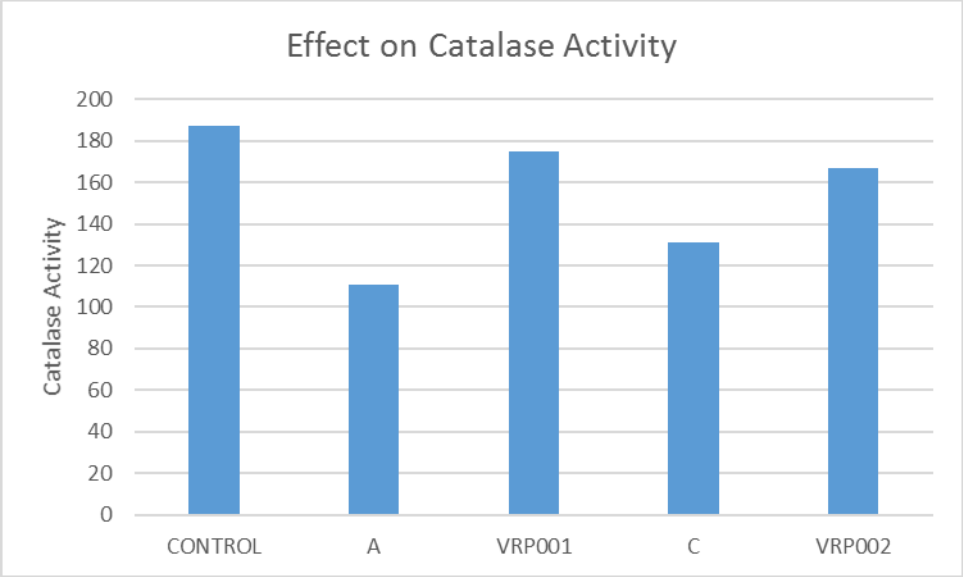

Figure 2: Effect on Catalase antioxidant enzyme activity

The administration of aminoglycosides A and C significantly decreased catalase antioxidant enzyme activity suggesting that the aminoglycosides induced oxidative stress could be cause of nephrotoxicity.

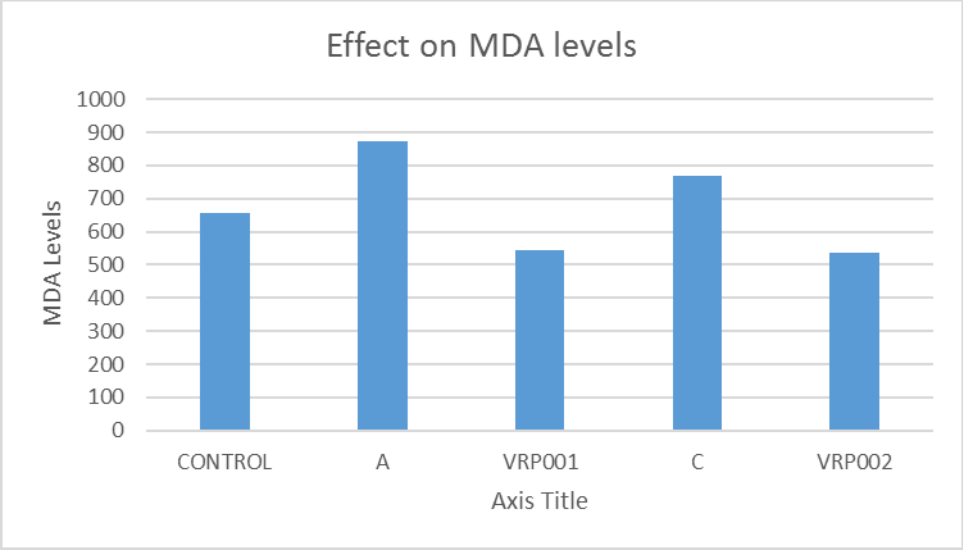

Figure 3: Effect on Malondialdehyde level

The administration of aminoglycosides A and C significantly increased MDA levels in the blood of treated mice and depress the antioxidant enzymes activity in kidney and heart.

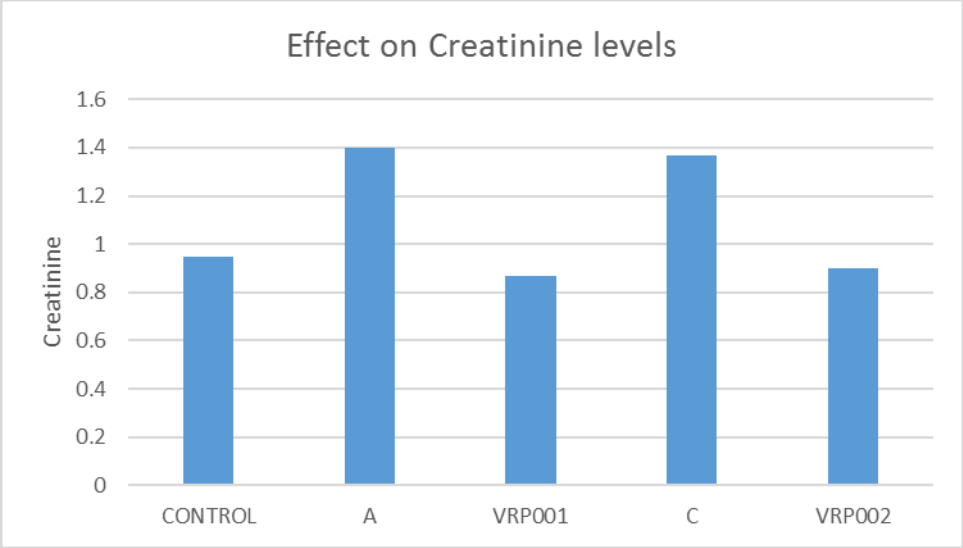

Figure 4: Effect on Creatinine level

The administration of aminoglycosides A and C significantly increased creatinine levels in the blood of treated mice and depress the antioxidant enzymes activity in kidney and heart.

CONCLUSION

Aminoglycosides are the common drugs which cause nephrotoxicity and ototoxicity. They alter antioxidant defense mechanism; generate free radicals, leading to tissue injuries such as nephrotoxicity and ototoxicity. They also increase cellular permeability by acting on membrane phospholipids, penetrate cell membrane of polyunsaturated fatty acids and cause tissue injury[4]. The administration of aminoglycosides significantly decreased SOD, catalase antioxidant enzyme activities and increased the MDA and creatinine levels in the blood of treated mice suggesting that the aminoglycosides induced oxidative stress could be cause of nephrotoxicity. They increased renal MDA levels and depress the antioxidant enzymes activities in kidney and heart. It was stated that oxygen free radicals were involved in aminoglycosides induced nephrotoxicity and singlet oxygen might directly inactivate the antioxidant enzymes[5].

Cephalosporins have free radical scavenging potential. They have low *in vitro* affinity for major chromosomally mediated lactamases and good stability against enzymatic hydrolysis. Cephalosporins protect against HOCL driven oxidative injury, possess cytoprotective properties but would not be expected to protect extracellular sulfhydryl group against free radical mediated oxidation[6].

The results of the present study conclude that fixed dose combination of cephalosporins and aminoglycosides prepared using chemical vector mediated technology possess antioxidant and free radical scavenging potential and contribute in improving the efficacy and safety profiles of these combinations.

Acknowledgement

We express our sincere gratitude to Principal Prof. Dr. Mathew George, HOD and PG In charge Prof. Dr. Lincy Joseph, Pushpagiri College of Pharmacy and Vikas Chaudhary for their support and guidance.

REFERENCES

1. Bennett, W.M. Elliot, W.C. Houghton, D.C. Gilbert, D. N. Defehr (1982). Reduction of experimental gentamycin in nephrotoxicity in rats by dietary calcium loading. Antimicrobial agents of chemotherapy. 22, 508-512.
2. Lapenna D, Cellini L, Gioia DS, (1995). Cephalosporins are scavengers of hydrochloric acid. BiochemPharmacol. 49: (9), 1249-1254.
3. Misra HP, Fridovich I. (1972) The role of superoxide anion in the autooxidation of epin Rephrine and a sample assay for superoxide dismutase. J Biol Chem(247) :3170-3175.
4. Bodmann KF (2005). Current guidelines for treatment of severe pneumonia and sepsis. Chemotherapy. 51, 227-233.
5. D Lew, D Pittet, F A Waldvogel (2004). Infections that complicate the insertion of prosthetic device. Hospital epidemiology and control. Lippincott Williams and Wilkins, Philadelphia.3rd edn . 1181-1205.
6. Hawkins, Keidel, W D Neff (1975). Sensory physiology. 3 (3); 707-748.