International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 5 (5): 307-312, 2009
ISSN 1811-7775

Diffusion of Sulbactomax and Ceftriaxone into Cerebrospinal Fluid of Meningitis Induced Rat Model

V.K. Dwivedi, M. Chaudhary, A. Soni, J. Yadav, A. Tariq, M.R. Siddiqui, A. Ahmad and P.S. Negi
Pre Clinical Division, Venus Medicine Research Centre, Hill Top Industrial Estate,
Bhatoli Kalan, Baddi, Hp-173205, India

Abstract: The present study was to investigate the comparative efficacy of ceftriaxone and a fixed dose combination of ceftriaxone plus sulbactam along with VRP1034 (Sulbactomax) in cerebrospinal fluid (CSF) of meningitis induced rat model. Eighteen rats were divided into three groups of six rats each. Meningitis were induced by MRSA strain (log 10^6 cfu mL^{-1}). Group I was infected group; whereas group II and III were ceftriaxone and sulbactomax treated groups. Drugs were analyzed in CSF by high performance liquid chromatography. Some biochemical parameters were studied in infected and treated groups. Present results showed that the mean level of ceftriaxone drug concentration was increased significantly in sulbactomax treated group in comparison to ceftriaxone alone treated group. Glucose level was increased in sulbactomax treated group as compared to ceftriaxone alone treated group. The levels of protein, calcium and phosphorus were significantly lowered in both treated group as compared to infected group. These biochemical parameters were decreased along with increased glucose level in sulbactomax treated group in comparison to ceftriaxone alone treated group. Present findings concluded that sulbactomax enhanced the penetration rate in CSF than ceftriaxone alone due to VRP1034. It plays a therapeutic role in crossing blood brain barrier and helps in prevention of bacterial meningitis infection.

Key words: Ceftriaxone, sulbactomax, cerbrospinal fluid, meningitis, blood brain barrier

INTRODUCTION

Bacterial meningitis is a common disease with high morbidity and mortality rate in the world wide (Sartz, 1984). Meningitis is an important clinical problem and it is characterized by an intense inflammatory response of sub arachnoid and ventricular space, breakdown of the Blood Brain Barrier (BBB), subsequent brain edema and vasculitis of the blood vessels (Schuchat *et al*., 1997; Tauber *et al*., 1997). The blood brain barrier is a physical and metabolic barrier that separates the peripheral circulation from the central nervous system and serves to regulate and protect the micro environment of the brain. It protects the integrity and function of the brain by selectively regulating the entry and exit of biologically important substances (Persidsky *et al*., 2006). Bacterial meningitis, a serious brain infection, can develop rapidly into a life-threatening infection even in healthy children or adults. Free radicals are also important factor for induction of bacterial meningitis (Van Furth *et al*., 1996).

The penetration of an antibacterial drug into Cerebral Spinal Fluid (CSF) is an important characteristic in term of its potential use in Central Nervous System (CNS) infections. Antibiotics either alone or in combination therapy are an important approach to address the management of chronic and acute diseases. Sulbactomax is a novel fixed dose combination of two drugs i.e., ceftriaxone and sulbactam along with a chemical vector (protected as trade secret) VRP1034. Ceftriaxone is third generation cephalosporin antibiotic. It has excellent activity against the common bacteria causing meningitis in children (Neu *et al*., 1981).

Ceftriaxone has been recommended by several authors for initial therapy of unidentified purulent meningitis in children and adults (Retsema *et al*., 1980; Labia *et al*., 1980). Several studies have reported that ceftriaxone has been shown to be additive or synergistic in combination with vancomycin, against several gram-negative pathogens (Chaudhary and Shrivastava, 2005; Tripta *et al*., 2007). Sulbactam is a molecule which is given in combination with the beta-lactam antibiotics to inhibit beta-lactamases, an enzyme produced by bacteria that destroys the antibiotics. When sulbactam is combined with ceftriaxone or other β-lactam antibiotic in a physical mixture, it restores their original activity both

Corresponding Author: Dr. Vivek Kumar Dwivedi, Venus Medicine Research Centre, Hill Top Industrial Estate, Bhatoli Kalan, Baddi, HP-173205, India Tel: 91-1795-302127 Fax: 91-1795302133

in vitro and *in vivo* (Retsema *et al*., 1980). Sulbactam is a β-lactamase inhibitor which combines with beta-lactam antibiotics to destroy their activity (Labia *et al*., 1980) and prevent the destruction of beta-lactamases, is under clinical development for co-administration with cephalosporin for treatment of infections. The activity of sulbactam plus cephalosporin against strain of *S. pneumoniae* and the enhanced penetration of sulbactam through inflamed meninges suggested that this combination might be useful in the treatment of bacterial meningitis in children (Hanninen and Rossi, 1986). In the present study, researchers have tried to evaluate the comparative efficacy of *in vivo* penetration of ceftraixone and Sulbactomax drug in cerebrospinal fluid of meningitis induced rats model.

MATERIALS AND METHODS

Chemicals: Tetrabutyl ammonium hydroxide (TBAH, AE 8AF58232), acetonitrile (SG8F80852) were purchased from Merck. Ceftriaxone and sulbactam standards were purchased from Zhejiang Yongning and Harbin pharmaceutical company Ltd, China. Biochemical kits for the determination of biochemical parameters were procured from Bayer Diagnostics India Ltd., Baroda, Gujrat India. Standard solution of ceftriaxone (25.0 mg) and sulbactam (12.5 mg) were prepared in 100 mL of distilled water. Sulbactomax was obtained from Venus Remedies Ltd. The ratio of active ceftriaxone to sulbactam used was 2: 1.

Bacterial strain: Methicillin-Resistant Staphylococcus Aureus (MRSA) bacterial strain maintained on nutrient agar slant were grown in septic culture in nutrient broth at 37°C for 24 h. Organisms were harvested and centrifuged at 2348 g for 15 min, washed three times and suspended in phosphate buffer saline (0.2 M, pH 7.0) to the desired concentration.

Meningitis model: For the purpose of this investigation, it was necessary to develop a model of experimental meningitis in the rat. Total eighteen rats (infected) were anesthetized intramuscularly with 10 mg kg^{-1} of ketamine (Ketolar; Parke-Davis, Prat de Llobregat, Spain).

Meningitis infection was induced in all the animals by direct intracisternal injection of 25 μL of saline containing log 10^6 cfu mL^{-1} MRSA strain via a 24-gauge needle. Meningitis infection was induced in rat within 7 to 10 days.

Animals and treatments: Total eighteen Wister rats, weighing 150 to 200 g were used in the experiment. They were housed at controlled temperature and humidity in an alternating 12 h light and dark cycle with free access to food and water. The study was approved by the institutional animal ethical committee. The rats were divided into three groups of six rats each. The drugs were given to animals intravenously according to their body weight for seven days treatment and CSF sample (0.05 to 0.12 mL) was collected from lumber puncture.

- **Group I:** Infected group (Saline treated group)
- **Group II:** Ceftriaxone treated group (103.3 mg kg^{-1} b.wt.)
- **Group III:** Sulbactomax (fixed dose combination of Ceftriaxone + Sulbactam+VRP1034) treated group (155 mg kg^{-1} b.wt.)

All the group of animals were sacrificed on 7th day and CSF sample was collected from lumber puncture.

Sample preparation for analysis: The CSF samples were collected from each group. After collecting the samples from each group added the 0.3 mL of chilled acetonitrile solution and mixed properly and left it for 10 min to precipitate the plasma proteins. Each sample was centrifuged at 5000 rpm for 20 min at 0-4°C and supernatant were aspirated out carefully for analysis of drug concentration.

Apparatus: Chromatographic separation was performed on Agilent 1200 series liquid chromatographic system equipped with G1311A quaternary pump, Agilent variable UV/visible detector and a G1329A auto injector. EZ Chrome Elite software was employed for data collecting and processing.

Chromatographic conditions: A buffer solution consisted of 50 mL of tetra butyl ammonium hydroxide (TBAH) in 1000 mL of distilled water was prepared and adjusted to pH 7.0 with ortho-phosphoric acid. The solvent used for the mobile phase was a mixture of buffer-acetonitrile (70:30). The mobile phase was passed through membrane filter (Millipore corp), 0.45 μm pore size and de-aerated under reduced pressure.

Ceftriaxone and sulbactam drug analysis: Ceftriaxone and sulbactum drugs were analyzed by the method of Shrivastav *et al*. (2009). For the analysis of Ceftriaxone and sulbactam concentration in CSF samples, To take 200 μL supernatant and added 150 μL of mobile phase and shaken vigorously. The chromatographic separation of ceftriaxone and sulbactam drug was performed by high performance liquid chromatography with a mobile phase containing buffer and acetonitrile. A column C-18 hypersil

ODS (5 µL, 4.6×250 mm) was used for the analysis of antibiotics. The flow rate and column temperature were maintained at 1.5 mL min^{-1} at 25°C respectively. After an equilibration of column with mobile phase for 2 h, 20 µL of sample was injected and detection of ceftriaxone and sulbactam antibiotics was performed at 220 nm UV wavelength. Under these chromatographic conditions, the retention time of ceftriaxone and sulbactam were found to be 5.2 and 3.3 min.

Biochemical analysis: Glucose, total protein, phosphorus and calcium parameters were analyzed in CSF sample by using a commercially available standard kit (Bayer Diagnostics India Ltd.,Baroda, Gujrat India).

Statistical analysis: The resulting data was analyzed statistically. All values are expressed in mean±SD. One-way Analysis of Variance (ANOVA) with student-Newman-Keuls comparison test was used to determine statistical difference between group II and group III. The $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The results of present study showed that there was statistically significant increase ($p<0.001$) in the mean level of (106.6±22.81 µg mL^{-1}) ceftriaxone of Sulbactomax treated group (group III) in comparison to ceftriaxone (39.45±3.28 µg mL^{-1}) alone treated group (group II). The mean level of sulbactam drug was also found to be increased (76.34±12.11 µg mL^{-1}; $p<0.001$) in Sulbactomax (ceftriaxone plus sulbactam with VRP1034) treated group (group III). The concentration of Sulbactomax drug (ceftriaxone plus sulbactam with VRP1034) was found to be increased in cerebrospinal fluid of meningitis induced rat in group III after treatment in comparison to administered ceftriaxone drug (group II) alone treatment.

The results are represented in Fig. 4. A typical Liquid chromatographic chromatogram of ceftriaxone and sulbactam standard is shown in Fig. 1, also Fig. 2 and 3 shows the ceftriaxone and sulbactam in CSF samples.

Glucose level was significantly increased ($p<0.001$) in CSF of ceftriaxone alone treated group as well as in Sulbactomax treated groups as compared with infected group (group I) after seven days treatment. When ceftriaxone alone treated group was compared to Sulbactomax treated group, the glucose level was significantly ($p<0.001$)found to be higher in CSF of Sulbactomax treated group after seven days treatment (Table 1).

Total protein level was significantly decreased ($p<0.001$) in the CSF of ceftriaxone alone treated group as well as in Sulbactomax treated group as compared with infected group (group I). When Sulbactomax treated group (group III) was compared with ceftriaxone alone treated group (group II), the protein level was found to be statistically ($p<0.001$) significant decreased in sulbactomax treated group after seven days treatment (Table 1).

Calcium and phosphorus levels were found to be decreased significantly ($p<0.001$) in CSF of ceftriaxone alone treated group (group II) and Sulbactomax treated groups (group III) as compared with infected group (group I) after seven days treatment. When these levels were compared after seven days treatment among group II and group III treated group, the levels of calcium and phosphorus were decreased significantly ($p<0.001$) in CSF of Sulbactomax treated group (group III) (Table 1).

Bacterial meningitis is the infection of the arachnoid membrane, sub arachnoid space and cerebrospinal fluid by bacteria. It is inflammation of the tissue covering the brain and spinal cord (the meninges). It is characterized by swelling of the meninges; increased pressure inside the skull blocks the flow of blood to the brain, starving the brain of nutrients and oxygen. Free radicals (super oxide, nitric oxide) are generated during meningitis infection (Van Furth *et al*., 1996; Neu *et al*., 1981).

Several studies have been reported that blood brain barrier is altered during meningitis in the rat (Townsend and Scheld, 1995; Kim *et al*., 1997). Reese and Karnovsky (1967) investigated that cerebral capillary endothelium as the major site responsible for blood brain barrier.

Increased permeability of the Blood Brain Barrier (BBB) is a pathological hallmark in several neurological disorders (Hawkins and Davis, 2005). Early and optimal treatment with antibiotics is the most effective intervention in bacterial meningitis. Sulbactomax is a fixed dose combination of ceftriaxone and sulbactam along with VRP1034 (a chemical vector). Ceftriaxone inhibits the mucopeptide synthesis in the bacterial cell wall.

Sulbactomax is a synergistic antimicrobial combination with marked *in vitro* antibacterial activity against a broad spectrum of organisms. Several studies have reported that cefotaxime and ceftriaxone were useful in the therapeutic concern of inflamed meninges (Nau *et al*., 1993).

Table 1: Effect of ceftriaxone and sulbactomax drug on some biochemical parameters in CSF of meningitis induced rat

Parameters (mg dL^{-1})	Infected control (Group I)	Ceftriaxone treated (Group II)	Sulbactomax treated (Group III)
Glucose	10.0±0.051	14.03±0.80[a]	19.83±0.25[a]
Protein	1.55±0.079	1.02±0.44[a]	0.89±0.15[a]
Calcium	7.58±0.42	5.12±0.98[a]	3.96±0.31[a]
Phosphorus	5.69±0.25	4.89±0.17[a]	2.09±0.31[a]

Values are expressed in Mean±SD. Statistical significant was determined between infected group (group I) vs. ceftriaxone alone treated group (group II) as well as Sulbactomax treated group (group III) and ceftriaxone alone treated group (group II) vs. Sulbactomax treated group (group III). Where [a]: ***p<0.001 (highly significant); [b]: **p<0.01 (significant), *p<0.05 (significant) and p>0.05 (not significant)

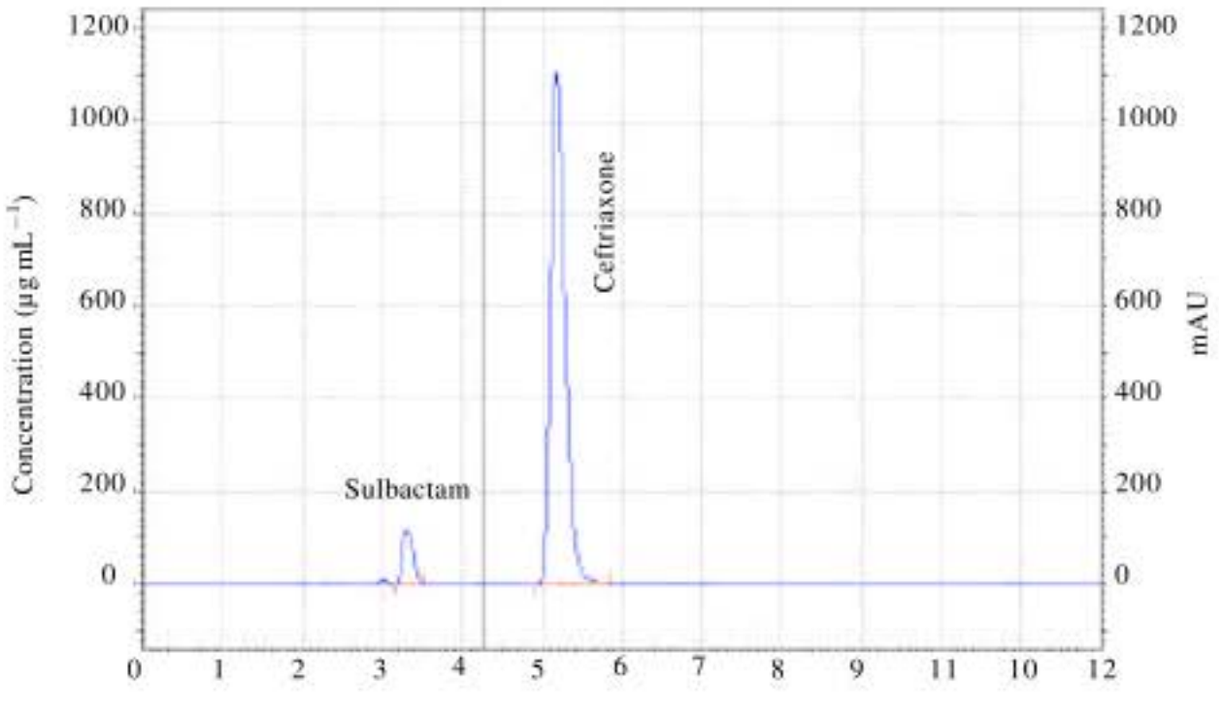

Fig. 1: Standard chromatogram of ceftriaxone and sulbactam. The retention time of sulbactam and ceftruaxone is found to be 3.3 and 5.2 min, respectively

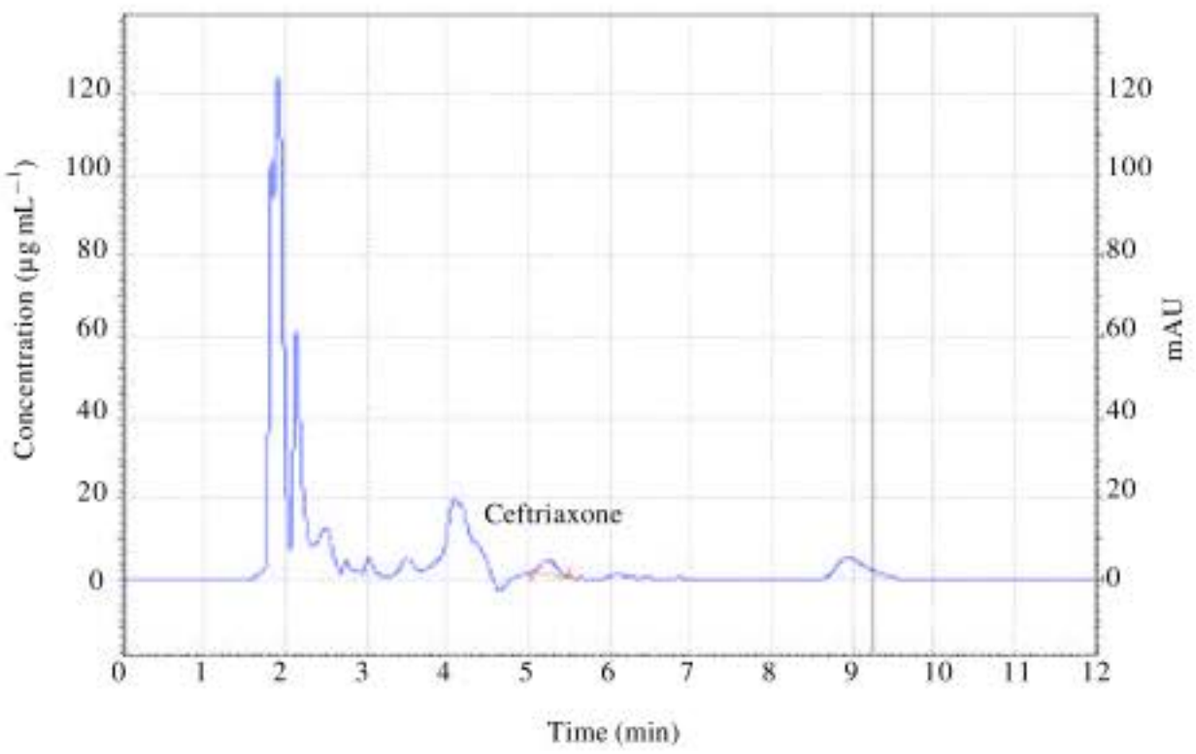

Fig. 2: Chromatogram of ceftriaxone in cerebrospinal fluid sample. The retention time of ceftriaxone is found 5.2 min which is similar to standard

In the present study, the mean concentration of ceftriaxone drug in CSF of meningitis induced rat of Sulbactomax treated group (group III) was found higher than ceftriaxone alone treated group (group II) due to synergistic effect of sulbactam and VRP1034 (Fig. 4). This is because when sulbactam is combined with ceftriaxone or other β-lactam antibiotic in a physical mixture, it restores their original activity both *in vitro* and *in vivo.*

Sulbactomax is active against all the organisms sensitive/resistant to ceftriaxone. In addition, it demonstrates synergistic activity (reduction in minimum inhibitory concentration for the combination versus those of each component) in a variety of organisms due to presence of sulbactam and VRP1034.

By forming a protein complex with beta lactamases, sulbactam irreversibly blocks their destructive hydrolytic activity. Sulbactam is used in combination with ceftriaxone and other beta-lactam antibiotics to enhance the spectra of microorganisms (Kucers *et al.*, 1987). The VRP 1034 is potent chelating agent which competes with microorganism for any of the trace iron and Ca^{2+} ions that are essential to the maintenance of their life cycle. It penetrates the cell membrane and open the Ca^{+2} Channel and enhanced the concentration of drug in the body. The role of VRP 1034 (trade secret) is to bind with essential divalent metal ions and hence make them unavailable to the bacteria for cellular replication and growth. The sensitivity of bacteria to VRP 1034 used to

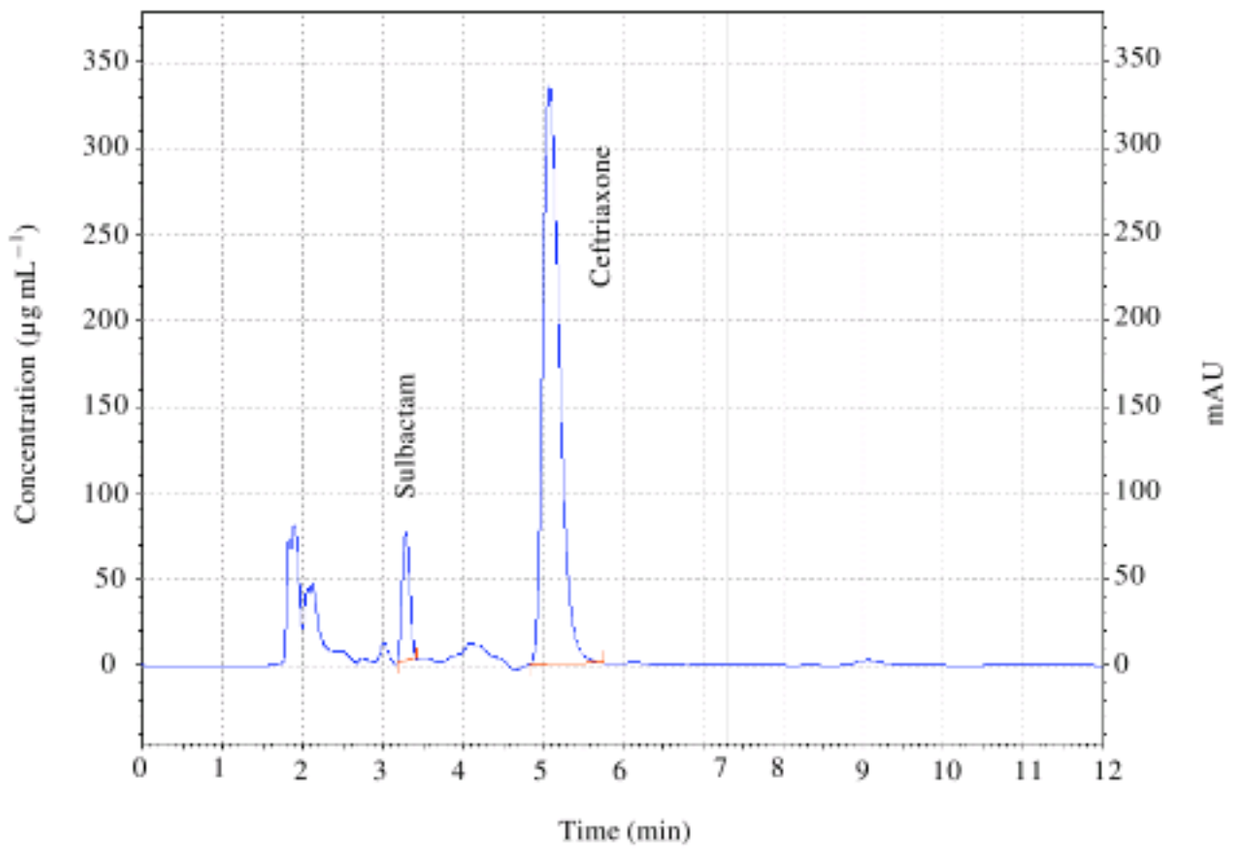

Fig. 3: Chromatogram of sulbactomax (ceftriaxone and sulbactam plus VRP 1034) during in CSF sample. The retentoin time of sulbactam and ceftriaxone was found to similar to standard

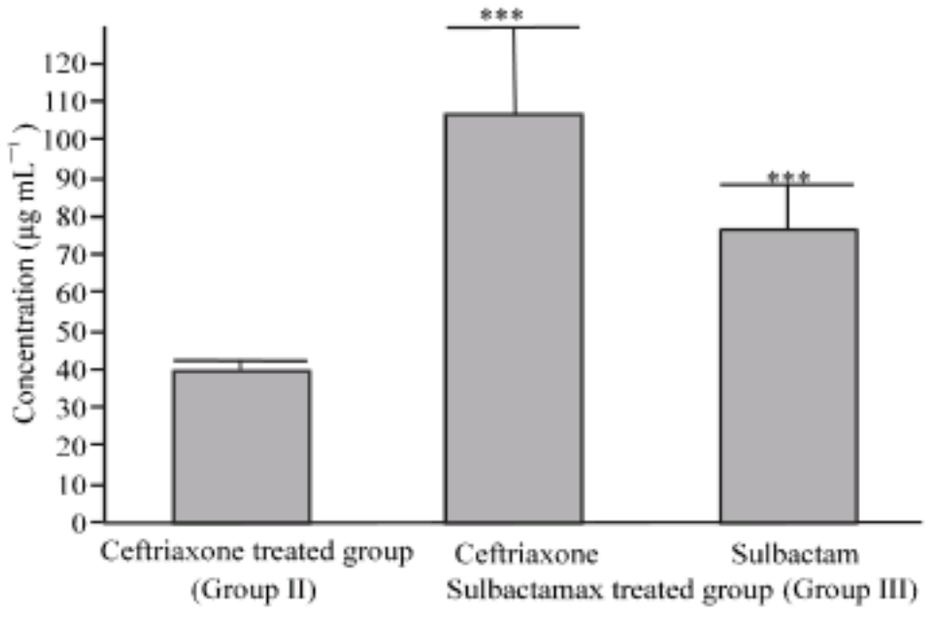

Fig. 4: Values are expressed in Mean±SD. Statistical analysis was determined between Ceftriaxone alone treated group (group II) vs ceftriaxone of Sulbactomax (ceftriaxone and sulbactam plus VRP1034) treated group (group III). $^{***}p<0.001$ (highly significant), $^{**}p<0.01$(significant), $^{*}p<0.05$ (significant) and $p>0.05$ (not significant)

enhance the succeptability of bacteria to antibiotics by destabilising the cell wall structure.

Increased permeability of the Blood Brain Barrier (BBB) is a pathological hallmark in several neurological disorders (Hawkins and Davis, 2005). During meningitis infection, free radicals are generated. Due to excessive generation of free radical, cell membrane may alter fluidity, enzyme activity and trans membrane ion fluxes. In the present study, the level of glucose was decreased in infected group (group I). When ceftriaxone alone and sulbactomax drug were administered to group II and group III (infected plus treated groups), the glucose level was significantly increased in both treated groups. When group II was compared with group III, the glucose level was found to be higher and statistically significant increased in Sulbactomax treated group (Table 1).

The protein, calcium and phosphorus levels were significantly increased in infected group. The level of total protein increased in CSF of infected group due to change in the permeability of blood brain barrier. Sulbactomax drug penetrates the cell membrane and open the Ca^{+2} Channel and enhance the concentration of drug in the body. Calcium is tightly regulated within the extracellular and intracellular compartments of the central nervous system, involving processes that include transport mechanisms across the Blood Brain Barrier (BBB) and cellular membranes, extensive binding by proteins and other macromolecules and sequestration within a variety of intracellular organelles. This data supported that blood brain barrier disruption was caused due to bacterial meningitis. After administration of both respective drugs for seven days treatment, the calcium, phosphorus levels were significantly decreased in both treated group. On comparison of ceftriaxone alone and Sulbactomax treated groups, the levels of these parameters were significantly decreased in Sulbactomax treated group.

CONCLUSION

The study concluded that a fixed dose combination of ceftriaxone and sulbactam with a chemical vector VRP1034 play a better therapeutic role than ceftriaxone

alone in bacterial meningitis. Sulbactomax also improved permeability of blood brain barrier than ceftriaxone alone treatment and assist in cure of meningitis caused by bacterial infection.

ACKNOWLEDGMENTS

Special thanks to Dr. Vijay Naithani for valuable suggestions. Authors are also thankful to management of Venus Medicine Research Centre for providing infrastructure and necessary grant for carrying out this study.

REFERENCES

Chaudhary, M. and S.M. Shrivastava, 2005. Minimum inhibitory concentration of ceftriaxone vancomycin combination and resistance development in *Staphyloccous aureus*. J. Natcon., 17: 382-384.

Hanninen, P. and T. Rossi, 1986. Penetration of sulbactam into cerebrospinal fluid in patients with viral meningitis or without meningitis. Rev. Infect. Dis., 8: 609-611.

Hawkins, B.T. and T.P. Davis, 2005. The blood-brain barrier/neurovascular unit in health and disease. Pharmacol. Rev., 57: 173-185.

Kim, K.S., C.A. Wass and A.S. Cross, 1997. Blood-brain barrier permeability during the development of experimental bacterial meningitis in the rat. Exp. Neurol., 145: 253-257.

Kucers, A., N. Bennett and R.J. Kemp, 1987. The Use of Antibiotics: A Comprehensive Review with Clinical Emphasis. 4th Edn., Williams and Wilkins, Philadelphia, Lippincott, p: 20.

Labia, R., V. Lelievre and J. Peduzzi, 1980. Inhibition kinetics of three R-factor mediated beta lactamases by a new beta lacam sulfone (CP-45899). Biochim. Biophys. Acta, 611: 351-357.

Nau, R., H.W. Prange, P. Muth, G. Mahr, S. Menck, H. Kolenda and F. Sörgel, 1993. Passage of cefotaxime and ceftriaxone into cerebrospinal fluid of patients with uninflamed meninges. Antimicro. Agents Chemother., 37: 1518-1524.

Neu, H.C., N.J. Meropol and K.P. Fu, 1981. Antibacterial activity of ceftriaxone (Ro 13-9904), a beta-lactamase-stable cephalosporin. Antimicro. Agents Chemother., 19: 414-423.

Persidsky, Y., S.H. Ramirez, J. Haorah and G.D. Kanmogne, 2006. Blood-brain barrier: Structural components and function under physiologic and pathologic conditions. J. Neuroimmune. Pharmacol., 3: 223-236.

Reese, T.S. and M. J. Karnovsky, 1967. Fine structural localization of a blood brain barrier to exogenous peroxidase. J. Cell. Biol., 34: 207-217.

Retsema, J.A., A.R. English and A.E.Girard, 1980. CP-45,899 in combination with penicillin or ampicillin against penicillin resistant *Staphylococcus*, *Haemophilus influenzae* and *Bacteroides*. Antimicrob. Agents Chemother., 17: 615-622.

Sartz, M.K., 1984. Bacterial meningitis-more involved than just thus the meninges. New Engl. J. Med., 311: 912-914.

Schuchat, A., K. Robinson, J.D. Wenger, L.H Harrison, M. Farley and A.L. Reingold, 1997. Bacterial meningitis in the United States in 1995. Active surveillance team. N. Engl. J. Med., 337: 970-976.

Shrivastav, S.M., R. Singh, A. Tariq, M.R. Siddiqui, J. Yadav, P.S. Negi and M. Chaudhary, 2009. A novel high performance liquid chromatography method for simultaneousdetermination of ceftraixone and sulbactam in sulbactomax. Int. J. Biomed. Sci., 5: 37-43.

Tauber, M.G. Y.S. Kim and S.L. Leib, 1997. Neuronal Injury in Meningitis. In: Defense of the Brain, Peterson P.K. and J.S. Remington (Eds.). Blackwell Science, Malden, Mass, pp: 124-143.

Townsend, G.C. and W.M. Scheld, 1995. *In vitro* models of the blood-brain barrier to study bacterial meningitis. Trends Microbiol., 3: 441-445.

Tripta, C., M. Shrivastava and S.M. Varughese, 2007. Studies of susceptibility and minimum inhibitory concentration of ceftriaxone and vancomycin alone and in combination in *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. J. Natcon., 19: 21-25.

Van Furth, A.M., J.J. Roord and R. Van Furth, 1996. Roles of pro-inflammatory and anti-inflammatory cytokines in pathophysiology of bacterial meningitis and effect of adjunctive therapy. Infect. Immu., 64: 4883-4890.