Research Article

Sub-acute toxicity study of fixed dose combination of ceftazidime - sulbactam in swiss albino mice

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ABSTRACT

The present investigation deals with sub-acute toxicity study of a fixed dose combination of Ceftazidime and sulbactam for injection in swiss albino mice at the dose levels of 30 mg/Kg, 60 mg/Kg and 120 mg/Kg body weight. Control was given only saline water. Sulbactam was added with ceftazidime in order to enhance the antimicrobial efficacy of ceftazidime injection which alone is insufficient to inhibit *Pseudomonas aeruginosa* and some other microbes completely. The mice (male and female both) were subjected to various haematological and biochemical investigations. The animals of both sexes from control and different dose groups exhibited normal body weight gain throughout the dosing period of 28 days. Ceftazidime and sulbactam did not show any significant feature of haematological & biochemical toxicity in any of the dose level in current study.

Key words: *Ceftazidime, Sulbactam, FDC, sub-acute toxicity*

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INTRODUCTION

Development of resistance in microorganisms to various antimicrobial agents, is becoming a matter of concern for clinicians all over the world [1-2]. Among a wide range of microbes, *Pseudomonas aeruginosa* is a leading cause of nosocomial infections [3] ranking second among the gram-negative pathogens and there are a limited number of antimicrobial agents with reliable activity against *P. aeruginosa*, including antipseudomonal penicillins and cephalosporins, carbapenems, and fluoroquinolones [4-6] The emergence of resistance in *P. aeruginosa* also limits future

therapeutic choices and is associated with increased rates of mortality and morbidity and higher costs. [6-9]

To overcome such type of problems, combination of 2 or 3 antibiotics have been suggested by various researchers [9-10]. It was concluded from these studies of emerging resistance that a combination of at least two antibiotics may provide better results by reducing resistance. Ceftazidime is a

third-generation cephalosporin antibiotic and possesses broad spectrum activity against gram- positive and gram-negative bacteria. It has been reported to be active against P*seudomonas aeruginosa and* is used in the empirical therapy of febrile neutropenia, in combination with other antibiotics. [11-12]

Sulbactam is a molecule which is given in combination with beta-lactam antibiotics to inhibit beta-lactamase, an enzyme produced by bacteria that destroys the antibiotics.[13-14] Hernández et al (2001) has reported the efficacy of sulbactam in experimental models caused by susceptible and intermediate *Acinetobacter baumannii* strains. Efficacy of sulbactam alone and in combination with ampicillin in nosocomial infections caused by multi- resistant *Acinetobacter baumannii* has been reported by Corbella et. al. [15]

One aspect of innovation of fixed dose combination of Ceftazidime - Sulbactam injection was to find better susceptible alternative of *P. aeruginosa* resistant to various antibiotics whereas other aspect was to provide more efficacious drug fulfilling all the therapeutic values which could not be provided by ceftazidime injection alone. Although toxicity studies on Ceftazidime and sulbactam alone are available. There are no reports on the toxicity of fixed dose combination of Ceftazidime and Sulbactam. The present study was designed to evaluate the total sub-acute toxicity study of fixed dose combination of Ceftazidime and Sulbactam. in swiss albino mice .

.MATERIAL AND METHOD

Animals

Forty eight (24 male and 24 female) healthy Swiss albino mice were divided into four groups of 6 mice per sex i.e., four dose groups receiving the dose of 30 mg/Kg, 60 mg/Kg and 120 mg/Kg body weight. Control group was given saline water only. Animals were provided with standard diet (pellets) supplied by M/s Ghosh Enterprise, Kolkata and aquaguard pure water was given *ad libitum*. They were housed in polycarbonate cages provided with bedding of husk. The temperature was maintained in between 20 to 24 °C and relative humidity between 30 to 70%; 12 hours each of dark and light cycle was maintained. They were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of dosing. They were assigned six per cage sex wise and the individual animal was fur marked with picric acid. The females were nulliparous and not pregnant.

Experiment Design and Drug Treatment

FDC of Ceftazidime and Sulbactam was given as intraperitonial injection at the dose levels of 30 mg/Kg, 60 mg/Kg and 120 mg/Kg in the dose volume of 1 ml/100 g body weight. The test article dissolve in water for injection were freshly prepared every day for 28 days. The control animals were administered vehicle only.

All the animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded. Physical parameters such as body weight, food consumption and water intake were monitored throughout the study. All the animals were observed twice daily for mortality during the period of the study. At the

end of the treatment, hematological and biochemical investigations were done to find out the toxic outcomes of Ceftazidime-Sulbactam.

Haematological Parameters

Blood samples were collected from orbital sinus following morning using heparin (22µl Heparin + 1 ml Blood Sample) as anticoagulant. Different hematological parameters such as haemoglobin%, reticulocyte%, hematocrit%, neutrophils%, lymphocytes etc. were studied using Sysmex^{TM}-K1000, Automated hematology Analyzer (Kobe, Japan).

Biochemical Parameters

Biochemical Parameters were performed in serum and plasma samples. All parameters were studied in Semi-Automatic Chemistry Analyzer (DR-7000E) Dirui Industrial Co., China, with Reckon Diagnostic Pvt. Ltd., Kit Baroda, India. Parameters done were total serum protein (Biuret Method, 2.5ml Reagent + 50 µl Sample), blood urea nitrogen (Berthelot Method 2.0ml Reagent + 10 µl Sample), serum glutamic pyruvic transaminase (UV Kinetic[IFCC Method] 1.0ml Reagent +100 µl Sample), serum glutamic oxaloacetic transaminase (UV Kinetic[IFCC Method] without pyridoxal phosphate (p-5'-p) 1.0ml Reagent +100 µl Sample), serum alkaline phosphatase (p-NPP, 1ml Reagent + 20 µl Sample) and blood sugar (GOD/POD Method 1.0ml Reagent + 10 µl Sample

Necropsy

All animals were sacrificed on day 29, using cervical dislocation technique under Ketamine anesthesia. Necropsy was carried out and the weights of following organs were recorded: liver, kidney and heart. The organ weights were recorded as absolute values and their relative values were calculated.

Statistical Analysis

Results are shown as Mean ± SD. Significance of difference between groups was evaluated using ANOVA. $P < 0.05$ was considered statistically significant. The study protocol for study was approved by "Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with Registration No. 148/bc/09/CPCSEA

RESULTS

All the animals were free of intoxicating signs throughout the dosing period of 28 days. No physical changes were observed throughout the dosing period. There was no significant change in the mean body weight of the animals in FDC treated groups as compared to vehicle treated control group at the end of treatment. Animals showed normal body weight gain throughout the dosing period of study in both male and female mice from control and different dose groups. No mortality was observed during the whole experiment.

During the dosing period and in the last day, the quantity of food and water intake by different dose groups was found to be comparable with control group. No abnormal deviations were observed. No significant changes were observed in the values of

different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits. There was no significant changes were observed in red blood cell (RBC), hemoglobin (Hb), serum protein, total leukocyte counts (TLC), hemocrit and platelet counts in all the treated groups as compared to respective control groups (Table1 & 2) Biochemical Investigations were performed to evaluate effect on liver and kidney function. All the biochemical parameters studied were found to be comparable with controls and were within the normal biological and laboratory limits. No significant changes were seen in BUN, creatinine, SGPT, SGOT and SAP levels in all the groups as compared to control group (Table 3 and 4). These proved no adverse effect on liver and kidney. The gross pathological and histopathological examination revealed no abnormality attributed to the treatment.

DISCUSSION

The current study presents the safety profile of a FDC of Ceftazidime- Sulbactam. Resistance of various microbes to antibiotics is an increasing clinical challenge and has become a matter of recognized public health threat [3, 9]. In an Italian study, lack of susceptibility (according to NCCLS breakpoints), was reported in meropenem, imipenem, carbenicillin, piperacillin, amikacin, ciprofloxacin, ceftazidime etc. while in ceftazidime, it was reported to be 13.4% and about half of the isolates (44.4%) were not susceptible to at least one of the antibiotics tested [5, 12, 18].

Leung et al (2008) has suggested the use of triple antimicrobial therapy (ceftazidime, amikacin, and sulbactam) for MDR *P. aeruginosa* infection in certain circumstances [14]. Combination of cefoperazone and sulbactam for treatment of intra- abdominal infections, has been suggested by Chandra et al [4] whereas successful treatment of a patient with multidrug resistant *Acinetobacter baumannii* meningitis with high dose ampicillin-sulbactam has been reported by Sayin et al (2008) [7]. Sulbactam efficacy in experimental models caused by susceptible and intermediate *Acinetobacter baumannii* strains also studied and proved [1,13,15].

Available reports clearly proves the rationale of sulbactam as beta- lactamase inhibitors and combining it with beta-lactams to increase susceptability [8,16-17,19]. All these studies suggest a combination of at least two antibiotics to get better results by reducing resistance. Our main aim was to assure the safety of, a FDC of Ceftazidime-Sulbactam. The results suggested, all the animals were free of intoxicating signs throughout the dosing period of 28 days and no mortality was observed during the whole experiment. Animals from control and the different dose groups exhibited normal body weight gain throughout the dosing period of 28 days in both male and female mice. During the dosing period and in the last day, the quantity of food and water intake by different dose groups was found to be comparable with that of control group. No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory

limits. Ceftazidime alone also proved safe and animal safety was reported.[6]

All the biochemical parameters studied were found to be comparable with controls and were within the normal biological and laboratory limits. The gross pathological examination revealed no abnormality attributed to the treatment. It appears to be established that combination regimen have not produced any deleterious effects on mice at all dose levels used in current study. This study provides clinically relevant data which can be utilised to decide the therapeutic safety of current dosage regimen. It can be concluded from the results of this study that a fixed dose combination therapy, may provide a safe alternative for beta lactamase producing resistant bacteria induced infections.

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Table 1: Effect of 28 days treatment with three doses of a FDC of Ceftazidime - Sulbactam on Hemogram in Male Mice

	Control	FDC of Ceftazidime-Sulbactam		
		30 mg/Kg	60 mg/Kg	120 mg/Kg
Hb (g%)	14.20±0.58	13.83±0.93	14.17±0.74	14.12±0.32
TRBC (X 10^6/mm^3)	7.52±0.69	6.96±0.27	7.37±0.41	8.86±0.52
Rt (%)	1.12±0.12	1.28±0.23	1.20±0.14	1.55±0.16
HCT (%)	43.22±0.94	42.35±0.57	42.68±1.07	46.23±1.04
MCV (μm^3)	52.95±1.53	53.53±1.21	134.00±19.96	54.18±2.54
MCH (pg)	17.23±0.67	17.82±0.66	17.62±0.57	17.17±0.49
MCHC (%)	33.72±0.88	33.98±0.67	33.48±1.14	35.02±2.08
Platelets (X 10/mm^3)	3.05±0.34	2.87±0.35	2.96±0.26	2.85±0.68
TWBC (X 10^3/mm^3)	6.93±0.74	7.10±0.58	7.52±0.53	6.35±0.59
N (%)	16.50±1.87	20.17±2.86	20.00±3.10	18.17±0.75
L (%)	80.50±1.64	75.00±1.41	76.50±3.15	80.00±7.72
E (%)	2.17±0.41	2.00±1.10	2.17±0.75	1.33±0.52
M (%)	0.83±0.75	0.97±0.98	1.03±0.82	1.10±1.17

Values are mean± SEM, n=6

Table 2: Effect of 28 days treatment with three doses of a FDC of Ceftazidime-Sulbactam on Hemogram in Female Mice

	Control	FDC of Ceftazidime-Sulbactam		
		30 mg/Kg	60 mg/Kg	120 mg/Kg
Hb (g%)	13.48±0.34	14.15±0.60	14.18±0.56	13.00±0.87
TRBC (X $10^6/m^3$)	7.36±0.69	7.35±0.53	6.84±0.75	7.27±1.14
Rt (%)	1.40±0.30	1.10±0.14	1.17±0.27	1.63±0.23
HCT (%)	42.70±1.31	42.80±0.95	42.77±0.94	43.73±1.32
MCV (μm^3)	52.87±1.01	53.43±1.25	53.73±1.58	52.13±1.56
MCH (pg)	17.52±0.80	17.42±0.65	17.70±0.53	17.17±0.49
MCHC (%)	33.97±0.92	33.85±1.20	33.77±0.96	34.03±0.89
Platelets (X $10^5/mm^3$)	2.91±0.23	2.82±0.33	3.25±0.32	3.42±0.32
TWBC (X $10^3/mm^3$)	7.42±0.73	7.38±0.54	6.86±0.59	7.32±0.34
N (%)	15.17±1.47	17.83±3.87	13.00±0.89	13.72±0.55
L (%)	78.83±1.94	78.83±3.71	81.33±3.39	85.67±1.97
E (%)	2.50±0.84	2.33±0.82	2.17±0.98	1.83±0.75
M (%)	1.83±1.33	0.93±1.33	0.98±1.60	1.05±0.98

Values are mean± SEM, n=6

Table 3: Effect of 28 days treatment with three doses of a FDC of Ceftazidime-Sulbactam on biochemical parameters in Male Mice

		FDC of (Ceftazidime-Sulbactam)		
	Control	30 mg/Kg	60 mg/Kg	120 mg/Kg
TSP (g%)	6.95±0.29	6.62±0.47	6.53±0.40	6.45±0.44
BUN (mg%)	41.50±1.87	41.33±3.01	41.83±1.72	42.83±1.47
SGPT (IU/L)	64.17±2.99	63.67±3.33	64.83±5.38	62.83±3.25
SGOT (IU/L)	106.50±4.76	109.67±7.99	104.50±9.01	101.00±9.44
SAP (IU/L)	364.50±23.65	345.00±17.55	347.67±8.55	351.83±9.20
BS (mg%)	95.67±4.13	96.33±5.01	97.17±5.23	93.33±2.73

Values are mean± SEM, n=6

Table 4: Effect of 28 days treatment with three doses of FDC of Ceftazidime-Sulbactam on biochemical parameters in Female Mice

	Control	FDC of (Ceftazidime-Sulbactam)		
		30 mg/Kg	60 mg/Kg	120 mg/Kg
TSP (g%)	6.88±0.40	6.73±0.50	6.52±0.48	6.22±0.27
BUN (mg%)	41.83±1.72	41.17±1.47	42.67±2.50	40.50±1.05
SGPT (IU/L)	66.00±4.47	64.17±3.87	65.00±3.29	63.83±3.31
SGOT (IU/L)	106.67±6.71	106.67±6.41	100.17±10.26	102.00±6.63
SAP (IU/L)	341.83±17.66	339.00±17.69	361.17±25.55	361.17±8.13
BS (mg%)	99.67±7.42	97.50±3.27	103.00±7.87	100.50±6.28

Values are mean± SEM, n=6

Abbreviations

TSP-Total serum protein, BUN - Blood urea nitrogen, SGPT – Serum glutamic pyruvic transaminase, SGOT- Serum glutamic oxaloacetic transaminase, SAP- Serum alkaline phosphatase, BS- Blood sugar., Hb-Haemoglobin, TRBC- Total Red blood cells, Rt – Reticulocyte, HCT-Hematocrit, MCV- Mean Corpuscular Volume, MCH- Mean Corpuscular Hemoglobin, MCHC- Mean Corpuscular Hemoglobin Concentration, TWBC- Total White blood cells, N- Neutrophils, L- Lymphocytes, E- Eosinophils, M- Monocytes