International Journal of Drug Development & Research
| July-Sept 2010 | Vol. 2 | Issue 3 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands

EVALUATION OF SUB-ACUTE TOXICITY PROFILE OF FIXED DOSE COMBINATION OF MEROPENEM AND SULBACTAM IN WISTAR RATS

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ABSTRACT

The present study investigated safety/toxicity profile of fixed dose combination of Meropenem and Sulbactam injection in rats at three dose levels, ranging from asymptomatic to high dose. The doses injected were 100mg/kg, 200 mg/kg and 400 mg/kg. Various physiological, hematological and biochemical parameters were studied. Outcomes of the present study suggested that no significant change in physiological, biochemical as well as hematological parameters were observed. No mortality was observed in any of the treatment groups. It was concluded that Meropenem and Sulbactam combination exerted no toxicity and is a safe medicine.

Key words: *Meropenem, Sulbactam, Toxicity*

Introduction

Growing bacterial resistance is major cause of concern for researchers as it contributes to increase in mortality, morbidity and treatment cost. *Acinetobacter baumannii*, a previously uncommon nonenteric gram-negative bacillus, has become a common nosocomial pathogen, especially in intensive care units [1]. It can colonize at multiple body sites of hospitalized patients and survive for a long time [2]. These characterstics contribute to its role in nosocomial infections [3]. The treatment options are very limited due to increased resistance.

Meropenem is an ultra-broad spectrum injectable antibiotic used to treat a wide variety of infections, including meningitis and pneumonia. Pathogens that are resitant to meropenem are generally resistant to commonly used antibiotics including penicillins, cephalosporins, monobactams, aminoglycosides and fluoroquinolones. Pathogens for which there are limited therapeutic options, can lead to a high fatality rate [4].

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 [5]. The addition of sulbactam, a beta-lactamase inhibitor, to meropenem has been reported to be effective against variety of resistant pathogens [4].

Keeping clinical significance of fixed dose combination of meropenem sulbactam in view, we plan to study its safety and sub acute toxicity profile in rats.

Materials and methods

Animals

Healthy *Wistar* rat of either sex were divided into four groups and assigned as three treatment groups and one control group. All groups consist of 6 male and 6 female animals. Animals were provided with standard diet (pellets) supplied by Amrut feed India and water was given *ad libitum*. They were housed in polyurethane cages (three in each) at controlled room temperature of 29 ± 2°C and a relative humidity of 50.5%, and a constant light-dark schedule (12 hours light and 12 hour dark cycle).

Animals were given freshly prepared intravenous injection of Meropenem Sulbactam for 28 days. The mixture of Meropenem Sulbactam was prepared in 0.9 % NaCl injection before administration and was injected at following dose levels; Group I –Control

group, Group II 100 mg/kg, Group III 200 mg/kg and Group IV 400 mg/kg.

Control group was injected 0.9 % NaCl only. Dosing was done approximately at the same time on each day. All the animals were observed for physical, biochemical and hematological alterations. Overnight fasted animals were sacrificed; blood and tissues samples were collected on 29th day. The Institutional animal ethics committee of Institute forToxicological Studies, Pune, India had approved the study protocol.

Hematological parameters

Hemogram was performed on ACT diff-2 Hematolgy Analyzer (Beckman Coulter India, Ltd., Mumbai, India).

Biochemical Parameters

Serum Gluatmic Oxaloacetic Transaminase (SGOT), Serum Gluatmic Pyruvic Transaminase activities (SGPT), Alkaline Phosphatase (ALP), Blood Urea Nitrogen (BUN) and plasma sugar levels were estimated on biochemistry analyzer using diagnostic kits (Transasia Biomedicals Ltd., Mumbai, India).

Histopathological examination

Liver, kidney, stomach, Heart and Lungs were removed from the sacrificed animals and were preserved in 10 % buffered formalin for histological examination.

Statistical analysis

Dunnett's test was used for the evaluation of data and $P < 0.05$ accepted as significant.

Results

Physical parameters

No behavioral changes were observed throughout the dosing period. No significant change group mean body weight was observed in all the groups as compared to control group on 29th day.

Hemogram

In male and female rat groups, no significant change was observed in hemoglobin (Hb), red blood cell counts (RBC), Rt (Reticulocyte), hematocrit (HCT), mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell corpuscular hemoglobin concentration (MCHC) ,white blood cell (WBC) counts and platelet counts in all the treated groups as compared to respective control groups (Table 1 & 2).

Table 1: Effect on Hemogram in male rats

Sr. No.	Dose mg/kg	Hb (%)	Total RBC ($x10^6$/cmm)	Rt (%)	HCT (%)	MCV μm^3	MCH(pg)	MCHC (%)	Platelets (10^5/cmm)	Total WBC) $x10^3$/cmm
I	Control	15.22 ± 1.72	5.55±0.53	1.65±0.46	52.00±5.55	66.92±8.09	20.27±3.71	33.18±2.97	5.40±1.74	6.63±1.23
II	100	15.02 ±1.65	6.17±0.88	1.68±0.29	50.17±6.82	63.43±7.99	18.97±2.91	31.73±2.14	5.35±1.28	7.30±1.04
III	200	12.74±0.73	5.83±1.04	1.75±0.31	47.67±4.23	60.20±6.35	16.89±0.70	32.35±1.80	6.56±0.80	6.43±0.76
IV	400	12.47±0.43	6.31±0.48	1.82±0.45	46.50±3.94	59.90±3.17	16.19±0.92	33.55±3.09	5.93±0.72	5.97±0.67

Values are repersented as Mean±SD, n=6.

Table 2: Effect on Hemogram in female rats

Sr. No.	Dose mg/kg	Hb (%)	Total RBC ($x10^6$/cmm)	Rt (%)	HCT (%)	MCV μm^3	MCH(pg)	MCHC (%)	Platelets (10^5/cmm)	Total WBC) $x10^3$/cmm
I	Control	16.43±1.22	5.90±0.53	1.45±0.41	54.00±5.62	63.57±6.07	21.03±4.11	32.37±2.27	6.20±0.53	6.92±1.14
II	100	13.20±0.88	5.95±0.86	1.70±0.36	50.33±7.34	62.80±7.38	17.88±1.87	32.83±2.62	5.85±1.14	6.43±0.76
III	200	12.96±0.70	6.07±0.67	1.70±0.49	45.50±5.32	60.04±3.25	16.35±0.71	32.64±2.33	6.22±0.71	6.13±0.69
IV	400	12.47±0.41	6.02±0.58	1.70±0.42	42.42±2.38	59.70±2.16	16.03±0.93	34.66±3.37	5.89±0.74	6.06±0.19

Values are repersented as Mean±SD, n=6.

Biochemical parameters

In male and female rat groups, no significant change in SGOT, SGPT and ALP activities were observed in all the treated groups as compared to respective control group. No significant change in Serum proteins and Blood sugar levels were observed in both the groups (Table 3 & 4).

Table 3: Effect on Biochemical parameters in male rats

Sr. No.	Dose mg/kg	TSP g%	BUN mg%	SGPT IU/L	SGOT IU/L	AP IU/L	Blood Sugar mg%
I	Control	7.75±0.29	33.80±2.32	89.52±8.82	91.50±5.01	402.33±96.67	95.48±6.37
II	100	7.65±0.29	30.82±2.61	67.67±11.72	92.83±8.11	447.00±49.25	98.40±7.22
III	200	7.60±0.27	30.41±2.90	96.03±6.86	94.87±8.58	412.67±53.05	100.23±4.89
IV	400	7.48±0.40	40.43±6.3	80.17±12.64	92.50±9.7	428.33±40.01	98.67±5.77

Values are repersented as Mean±SD, n=6.

Table 4: Effect of Biochemical parameters in female

Sr. No.	Dose mg/kg	TSP g%	BUN mg%	SGPT IU/L	SGOT IU/L	AP IU/L	Blood Sugar mg%
I	Control	7.74±0.42	32.78±3.83	71.17±15.42	89.67±8.55	420.33±37.73	97.98±3.91
II	100	7.59±0.43	30.78±4.12	66.83±12.54	92.00±6.26	413.17±35.10	94.77±5.96
III	200	7.53±0.46	30.48±1.98	86.00±14.56	90.53±6.43	428.17±48.44	97.90±3.27
IV	400	7.40±0.35	31.23±3.12	80.00±12.13	88.33±6.68	419.17±29.78	100.90±5.69

Values are repersented as Mean±SD, n=6.

No significant treatment related histopathological changes were observed up to the dose of 400 mg/kg body weight.

Discussion

The β-lactams antimicrobial agents consist of four major groups: penicillins, cephalosporins, monobactams and carbapenems. The β-lactam ring of these antibiotics can be hydrolysed by β-lactamases [6.7]. Bacteria generate resistance to antimicrobial agents by producing β-lactam hydrolyzing enzymes (β-lactamases) or mutated types of penicillin binding proteins [8, 9]. Sulbactam is a established β-lactamase inhibitors that can be combined with β-lactam antibiotics to prevent their hydrolysis by β-lactamases [7]. Sulbactam thus makes β-lactam antibiotics effective even against many resistant strains. In the present study, we studied safety profile of the potential fixed dose combination meropenem and sulbactam in rats.

Meropenem is an ultra-broad spectrum injectable antibiotic used to treat a wide variety of infections, including meningitis and pneumonia. It is a beta-lactam and belongs to the subgroup of carbapenem, similar to imipenem and ertapenem. It inhibits bacterial wall synthesis like other beta-lactam antibiotics [10].

Liver and kidneys play significant roles in various metabolic processes. In addition, liver plays xenobiotic function, while kidneys are sites for filtration and reabsorption. Thus effect of FDC on function of these organs was studied. Major part of elimination of meropenem sulbactam takes through renal excretion [11]. In the present study increase in body weights and growth of treated animals of both the sex were followed similar pattern as in respective control groups. There were no signs of injury at site of injection in all the groups. Hematological parameters were studied in all treated as well as control groups and results had shown no abnormal effect on hematological parameters in treated animals as compared to respective control group.

Impact of the FDC was also evaluated on liver function tests. No significant changes were observed in liver function parameters such as SGOT, SGPT and SAP activities in meropenem sulbactam treated all groups of either sex as compared to the respective control group which confirmed lacking in hepatotoxic potential of FDC.

No significant difference in renal function tests were observed on treatment with the FDC of meropenem and sulbactam as compared to respective control groups ruling out any nephro toxic potential.

Histopathological analysis were done and have also supported safety of meropenem sulbactam data infered from physiological, biochemical and heamatological parameters. There were no signs of toxicity observed in any of the organs in meropenem sulbactam treated animals at all the three doses as compared to control.

In conclusion, fixed dose combination of meropenem and sulbactam was safe in rats even at very high dose levels.

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Article History:----------

Date of Submission: 02-04-10

Date of Acceptance: 08-05-10

Conflict of Interest: None

Source of Support: Nil