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Assessment of Sub-Chronic Injectable Toxicity of Combination of Meropenem and Sulbactam in Mice

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ABSTRACT

Meropenem is a broad spectrum antibiotic belonging to β-lactam group of antibiotic and sulbactam is β-lactamase inhibitor. Combination of the meropenem and sulbactam can be used for infections caused by microorganisms which are resistant to meropenem and thus increase the activity of meropenem towards resistant strains. The purpose of this study was to investigate safety and toxicity effects of intravenous administration of combination of meropenem and sulbactam. In present study, mice were administered dose of 100 mg/Kg, 200 mg/Kg and 400 mg/Kg of combination of meropenem and sulbactam for 28 consecutive days and toxic effects were assessed using biochemical, hematological and histology of vital organs. No mortality or toxicity effects were observed during the course of study. Various physiological, hematological and biochemical parameters were studied. No damaging consequences were observed in physiological, biochemical and hematological parameters. The observation gave the good evidence of a favorable safety profile of combination of meropenem and sulbactam.

Keywords: Meropenem, Sulbactam, Toxicity.

INTRODUCTION

The use of antibiotics in clinical practice has become extraordinarily common. There are several well known potential and actual justifications for the concomitant use of more than one antibiotic. The necessity of providing initial broad antibacterial coverage in critically ill patients in sepsis of unknown etiology has lead to development of synergistic combinations of antibiotics. [1] In addition, antibiotic combinations are commonly prescribed for the treatment of infections due to more than one organism. They can also be combined in order to minimize resistance to individual agents. [2] The actual choice of agents depends on the critical clues as to the nature of infecting organism and on patterns on antimicrobial sensitivity among the bacteria. Combination

of β-lactam antibiotic and β-lactamse inhibitor has been successful in circumventing the bacterial evolutionary drive towards resistance. [3]

Meropenem is a β-lactam antibiotic belonging to carbapenem class. It has an ultra-broad spectrum of activity. Pathogens that are resitant to meropenem are generally resistant to commonly used antibiotics including penicillins, cephalosporins, monobactams, aminoglycosides and fluoroquinolones. Meropenem therapy is an attractive choice for the empirical treatment of mild to moderate bacterial infections. [4] Sulbactam is a semi-synthetic penicillinate sulfone containing a β-lactam ring. It is an irreversible inhibitor of wide variety of β-lactamases. Combination of meropenem and sulbactam has been reported to have synergistic effect. [5]

The aim of the present study was to investigate the acute and sub-chronic toxicity effects of the combination of Meropenem and Sulbactam, as well as the possible effects on biochemical, hematological and histological parameters.

MATERIALS AND METHODS

Animals

Young albino mice of both sexes, were obtained from the animal house of Indian Institute of Chemical Biology, Kolkata. All animals were held in quarantine for 7 days for acclimatization and to document their health status before their definitive inclusion into the experiment. An institutional independent board, Institutional Animal Ethical Committee, Jadavpur University, Kolkata; approved the study protocol and the animal use for this study. The study protocol was in consistent with Guidelines for Testing of Chemicals, the Good Laboratory Practice (GLP) guidelines and Regulations for the Use of Laboratory Animals, which are in consistent with international ethical regulations for the management of laboratory animals.

Animals were housed 6 each, of the same sex in polycarbonate cages provided with bedding of husk. Animals were maintained in a filter protected air-conditioned room, at a controlled temperature of 20 to 24 °C and relative humidity between 30 to 70 %. Twelve hours each of dark and light cycle was maintained. Mice were fed with standard diet (pellets) which was supplied by M/s Ghosh Enterprise, Kolkata. Animals were freely accessed to aqua guard pure water in glass bottles *ad libitum*.

A total of forty eight rats i.e. 24 male and 24 female healthy mice were used for study. Animals were divided into four groups of 6 rats per sex i.e. four dose groups receiving the dose of 0 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg. The individual animals were fur marked with picric acid. The females were nulliparous and not pregnant.

Test substance, administration and dosage

Once concluded the quarantine period, animals were categorized into 4 groups of each sex: a control group (0 mg/kg) and three other test groups (100 mg/kg, 200 mg/kg, 400 mg/kg). Treatments were given once a day by intravenous route. 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 the following dose levels; ; Group I –Control group, Group II 100 mg/kg, Group III 200 mg/kg and

Group IV 400 mg/kg. The test article suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Overnight fasted animals were sacrificed; blood and tissue samples were collected on 29th day.

Observations

Symptoms

All animals were observed daily for clinical signs. The time of onset, intensity and duration of symptoms were recorded.

Mortality

All animals were observed twice daily for mortality during the period of study.

Body weight

The weight of each rat was recorded on day zero and at weekly intervals throughout the course of the study. The groups mean body weights were calculated.

Food consumption

The quantity of food consumed by groups consisting of six rats each was recorded weekly and the food consumption per rat was calculated for control and dose groups.

Laboratory Investigations

On completion of the dosing period of 28 days, animals were fasted overnight and blood samples were collected from orbital sinus following morning using heparin as anticoagulant.

Hematological parameters

Hematological parameters were studied using Sysmax-K1000 Cell Counter.

Biochemical parameters

Serum Gluatmic Oxaloacetic Transaminase (SGOT), Serum Gluatmic Pyruvic Transaminase (SGPT), Serum Alkaline Phosphatase (SAP), Blood Urea Nitrogen (BUN) and plasma sugar levels were estimated on biochemistry analyzer using diagnostic kits (Robonik ASP-300).

Histopathological examination

Organs from control and animals treated at the highest dose level of 400 mg/kg were preserved in 10 % formalin for histopathological examination. Heart, Kidneys, Liver, Lungs and Stomach of low and intermediate dose group animals were preserved for possible histopathological examination.

Statistical analysis

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

RESULTS

Physical parameters

Animals from control and the different dose groups exhibited normal body weight gain throughout the dosing period of 28 days. No significant change in mean body weight was observed in all the groups as compared to the control group on 29th day.

Food consumption

During the dosing period and at the termination the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

Hematological studies

On completion of 28 days treatment, overnight fasted animals were sacrificed and studied for various hematological parameters. No significant changes were observed in the values of different parameters studied when compared with control and the values obtained were within normal biological and laboratory limits as discussed in table 1 and 2.

Table 1: Effect on Hemogram in male rats

Gr. 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	13.20 ± 1.39	6.53±0.45	1.27±0.30	39.93±1.77	71.03±1.91	17.08±1.90	30.05±2.25	8.17±0.54	6.36±0.92
II	100	13.27 ±1.12	6.26±0.91	1.37±0.29	42.40±3.29	64.12±2.46	17.33±2.45	30.08±2.92	7.63±1.08	6.53±0.82
III	200	12.37±1.69	6.28±0.98	1.55±0.55	43.88±4.15	60.45±5.99	17.43±1.89	25.87±3.95	7.31±0.70	5.72±0.69
IV	400	12.53±1.47	6.51±0.70	1.25±0.21	39.65±3.51	60.35±5.61	19.42±1.46	28.88±5.38	7.14±0.99	6.12±0.86

Values are repersented as Mean±SD, n=6. Hb (Hemoglobin), RBC (Red Blood Corpuscles), Rt. (Reticulocyte), HCT (Hematocrit), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemogl;obin Concentration), WBC (White Blood Corpuscle)

Table 2: Effect on Hemogram in female rats

Gr. 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	12.88 ± 1.16	6.19±0.37	1.27±0.38	40.60±2.01	57.38±4.91	21.28±4.64	26.65±3.29	8.60±0.66	6.83±0.56
II	100	12.17 ±2.08	6.19±0.46	1.62±0.53	41.37±2.33	65.45±4.97	18.88±1.73	31.25±3.37	7.84±0.80	6.39±0.57
III	200	12.62±1.26	6.46±0.79	1.37±0.38	40.15±6.12	64.88±3.91	19.38±1.64	27.47±4.95	7.56±0.91	6.49±0.59
IV	400	11.92±1.80	6.37±0.52	1.42±0.50	40.60±6.07	61.00±5.44	17.47±2.06	28.48±5.05	7.68±1.11	6.23±0.67s

Values are repersented as Mean±SD, n=6. Hb (Hemoglobin), RBC (Red Blood Corpuscles), Rt. (Reticulocyte), HCT (Hematocrit), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemogl;obin Concentration), WBC (White Blood Corpuscle)

Table 3: Effect on Biochemical parameters in male rats

Gr. No.	Dose (mg/kg)	Total Serum Protein (g%)	BUN (mg%)	SGPT (IU/L)	SGOT (IU/L)	SAP (IU/L)	Blood Sugar (mg%)
I	Control	6.53±0.70	22.33±2.16	41.00±3.79	57.50±4.93	265.67±39.00	99.17±4.83
II	100	6.62±0.86	24.00±4.20	41.17±1.94	57.33±4.18	289.83±32.11	99.50±5.47
III	200	6.77±0.34	24.33±2.34	41.67±3.20	59.83±4.79	309.00±17.84	102.17±2.86
IV	400	6.75±0.38	25.50±3.08	43.33±4.93	68.83±3.76	340.83±55.71	108.17±2.79

Values are repersented as Mean±SD, n=6. BUN (Blood Urea Nitrogen), SGPT (Serum Glutamic Pyruvic Transaminase), SGOT (Serum Glutamic Oxaloacetate Transaminase), SAP (Serum Alkaline Phosphatase)

Biochemical parameters

All the biochemical parameters studied i.e. Total serum protein; SGPT, SGOT, SAP, BUN and Blood sugar were found to be comparable with controls and were within the normal biological and laboratory limits.

Table 4: Effect on Biochemical parameters in female rats

Gr. No.	Dose (mg/kg)	Total Serum Protein (g%)	BUN (mg%)	SGPT (IU/L)	SGOT (IU/L)	SAP (IU/L)	Blood Sugar (mg%)
I	Control	5.75±0.55	22.33±2.42	41.17±2.32	56.67±4.72	236.33±27.44	100.00±4.98
II	100	6.68±0.49	24.00±2.97	42.17±4.71	61.33±2.58	257.00±44.27	93.67±5.28
III	200	5.92±0.34	21.83±3.31	40.50±1.87	54.50±3.39	300.83±13.29	99.50±5.61
IV	400	6.30±0.84	28.33±4.37	54.33±3.61	59.33±3.61	337.17±41.60	103.83±3.54

Values are repersented as Mean±SD, n=6. BUN (Blood Urea Nitrogen), SGPT (Serum Glutamic Pyruvic Transaminase), SGOT (Serum Glutamic Oxaloacetate Transaminase), SAP (Serum Alkaline Phosphatase)

Histopathological parameters

Animals from control and the different dose groups exhibited normal histopathological parameters throughout the dosing period of 28 days.

Table 5: Histopathological parameters in male rats

Gr. No.	Dose (mg/kg)	Body Weight (g)	Liver (g)	Kidneys (g)	Heart (g)
I	Control	27.43±1.15	2.82±0.22	0.40±0.02	0.19±0.02
II	100	26.72±1.36	2.79±0.28	0.41±0.02	0.23±0.02
III	200	26.90±0.92	3.06±0.23	0.41±0.06	0.21±0.02
IV	400	27.63±2.08	2.77±0.36	0.46±0.03	0.22±0.03

Table 6: Histopathological parameters in female rats

Gr. No.	Dose (mg/kg)	Body Weight (g)	Liver (g)	Kidneys (g)	Heart (g)
I	Control	25.03±1.20	2.83±0.55	0.41±0.03	0.21±0.02
II	100	26.32±1.02	3.11±0.44	0.42±0.03	0.23±0.02
III	200	26.38±1.01	2.92±0.42	0.40±0.02	0.22±0.02
IV	400	25.37±1.22	3.01±0.30	0.43±0.04	0.21±0.02

DISCUSSION

It has been recognized that there is a clinical need for novel β-lactam/β-lactamase combinations with the appropriate broad spectrum. [6, 7] Meropenem is a new carbapenem antibiotic and is active against large number of gram positive bacteria, penicillinase producing bacteria and methicillin susceptible staphylococci but some β-lactamase producing strains are resistant to Meropenem. [8, 9] Sulbactam is a β-lactamase inhibitor which requires concomitant administration of a β-lactam antibiotic for potential kinetic interaction with bacteria. [10] Sulbactam makes β-lactam antibiotics effective even against resistant strains. Meropenem (β-lactam antibiotic)-Sulbactam (β-lactamse inhibitor) combinations were well tolerated and have good safety profiles.

This study demonstrates that combination of meropenem and sulbactam administered intravenously to mice for a month did not show evidences for toxicity and was consistent with lack of sub-chronic toxicity. The overall mortality rate (0/48) gave an indication of the lack of toxicity of combination even at highest dose of 400 mg/kg.

The hematological parameters did not show a significant difference between control and treated animals. This data suggested that the combination of meropenem and sulbactam can be safely used without effecting hematological parameters.

The biochemical parameters studies were comparable with controls and were within normal biological and laboratory limits. It supported the authenticity of our experimental focus and non sensitivity of this mice strain for sub-chronic toxicity.

The histopathological examination of animals from high dose group revealed no abnormality attributable to the treatment.
The design and objectives of this study was far successful in demonstrating a potential of combination of Meropenem and Sulbactam without producing toxic effects.

CONCLUSION

The extensive antimicrobial activities and clinical efficacies comparable to standard therapies and good safety records make the combination of meropenem and sulbactam as an effective agent for the treatment of hospitalized patients with severe infections, especially where there is suspicion of polymicrobial infection or resistant strains.

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