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Sub-Acute Toxicity Study of Fixed Dose Combination of Sulbactomax (Ceftriaxone-Sulbactam) in Swiss Albino Mice and Wistar Rat

A. Tamta and M. Chaudhary
Intellectual Scientific Division, Venus Medicine Research Centre,
Hill Top Industrial Estate, Bhatoli Kalan, Baddi, H.P-173205, India

Abstract: The present study investigated safety/toxicity profile of Sulbactomax (Ceftriaxone-Sulbactam for injection), a fixed dose combination, in *Mus musculus* mice and SD rats at three dose levels, 10, 50 and 150 mg kg^{-1} ranging from asymptomatic to high dose. Sulbactomax was introduced in order to enhance the antimicrobial efficacy and to combat resistance towards beta-lactamase producing bacteria. The combination has been reported to be highly effective as well as synergistic for many resistant strains and carry the potential for its usage in empirical therapy for various bacterial infections. To establish the safety profile of combination, 28 days repeated dose sub-acute toxicity study was conducted on mice and rat (male and female). Various hematological parameters were studied in addition to physiological and biochemical parameters in order to study toxicity profile of Sulbactomax. There were no signs of toxicity observed at any of the dose levels used in this study. Animals from control and different treated groups exhibited normal body weight gain throughout the dosing period of 28 days. No mortality was observed in any of the treatment groups during the course of whole study. Hematological as well as biochemical parameters were unaltered at all three dose levels in Sulbactomax treated rat and mice. From the present study, it can be concluded that Sulbactomax (the fixed dose combination of Ceftriaxone -Sulbactam) is safe even at the dose level which is several folds of the intended human dose.

Key words: Sub-acute toxicity, Sulbactomax, cephalosporins, beta-lactamas

INTRODUCTION

At present bacteria, responsible for community-acquired infections have developed resistance to many of the antibacterial agents which are commonly used, particularly to beta-lactams (Carmeli *et al*., 1999; Lambert, 2002). This emerging problem was the main motivation to release, ceftriaxone, an extended spectrum cephalosporin, in the clinics for the treatment of severe infections or infections caused by multiple-resistant strains (Ogtrop *et al*., 1990; Bonfiglio *et al*., 1998; Li *et al*., 1994). In the current scenario several documented evidence point towards the development of resistance to extended-spectrum cephalosporins including ceftriaxone in bacteria isolated from patients with many infection including nosocomial infections (Caron *et al*., 1990; Sauve *et al*., 1996). The antibacterial activity of Ceftriaxone is due to the inhibition of cell wall synthesis (Goldstein *et al*., 1995). It has a high degree of stability in the presence of beta-lactamases, both penicillinases and cephalosporinases of gram-negative and gram positive bacteria. However, chromosomally

Corresponding Author: A. Tamta, Intellectual Scientific Division, Venus Medicine Research Centre, Hill Top Industrial Estate, Bhatoli Kalan, Baddi, H.P-173205, India

mediated enzymes can be induced in some bacteria which can lead to the development of resistance to Ceftriaxone (Jacoby and Medeiros, 1991; Goldstein *et al*., 1993; Kitzis *et al*., 1988; Briñas *et al*., 2005). This strongly emphasizes the need for more effective compounds or combination which might help to overcome the resistance. Sulbactam was approved recently in many countries in combination with beta-lactam antibiotics, including India (Levin, 2002). Sulbactum is a potent and highly specific inhibitor of a wide variety of beta-lactamases produced by common Gram-negative and Gram-positive aerobes and anaerobes (Bhattacharjee *et al*., 2008). Sulbactum forms a protein complex with beta-lactamases, thereby irreversibly blocking their destructive hydrolytic activity (Betrosian *et al*., 2008). Thus, the full potential of Ceftriaxone against *Enterobacter* and *Pseudomonas* species is restored by the addition of Sulbactam (Corbella *et al*., 1998).

This combination, Sulbactomax (Ceftriaxone-Sulbactam) was developed as an anti-infective therapy (Shrivastava *et al*., 2009; Levin, 2002). Ceftriaxone is a broad-spectrum semi-synthetic third-generation cephalosporin with a potent bactericidal activity against a wide range of gram-positive and gram-negative bacteria (Carmeli *et al*., 1999). Combination of sulbactam with beta-lactam antibiotics have been used successfully for the therapy of infections caused by organisms resistant to the antibiotic alone (Levin, 2002). In spite of its potential role in antimicrobial therapy there is a big lacuna in toxicity studies of this combination. The aim of the current study was to delineate the toxicity profile of Sulbactomax and determine its safety profile in animal study.

MATERIALS AND METHODS

Animals

Mice weighing between 20-25 g and rats weighing between 150-160 g were selected for the study and were divided into four groups (three treatment groups and one control group). The selected animals were separated on the basis of sex and were assigned to the requisite experimental groups. Each experimental group consisted of twelve animals, six of each sex. The groups were assigned to the animals by randomization technique using a random distribution table. Each treatment group and control consists of 12 animals (6 males and 6 females).

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 25±2°C and a relative humidity of 50.5% ±5 and a constant light-dark schedule (12 h light and 12 h dark cycle). The study was carried out from 5 March 2007 to 31 June 2007 at Venus Medicine Research Centre, Panchkula, India.

Reagents

All chemicals were purchased from Sigma, St. Louis, MO, USA. Sulbactomax (Ceftriaxone Sulbactam combination) was procured from Venus Remedies Limited Baddi (India).

Experimental Design

The drug preparations of Ceftriaxone Sulbactam were made in sterile water just before use. Sterile water was injected intravenously to the animals vehicle treated groups which served as sham treatment.

The drug was administered at three doses (see dosage chart) with corresponding controls for twenty eight days. The animals were divided into four groups with control and each experimental group was administered with the drug at doses given below:

A control group was also maintained comprising of 6 male and 6 female animals. Sterile water was injected to the animals of control group.

Treatment was done once daily for 28 days. The study protocol for study was approved by Institutional Animal Ethics Committee (IAEC) of Institute for Toxicological Studies, Pune, India.

	No. of animals used		Repeated dose Sulbactomax	
Groups	Male	Female	Mice	Rats
Control	6	6	Sterile distilled water/animal	Sterile distilled water/animal
Low dose	6	6	10 mg/kg/day	10 mg/kg/day
Intermediate dose	6	6	50 mg/kg/day	50 mg/kg/day
High dose	6	6	150 mg/kg/day	150 mg/kg/day

Physical Parameters

Physical parameters (body weight, food and water intake) and local injury were studied throughout the treatment. Mortality if any, in all the groups, during the course of treatment was also recorded. At the end of treatment haematological, biochemical (liver function tests and renal function tests) and histological parameters were studied. The organs were quickly blotted, weighed on digital balance and processed for histological studies.

Haematological Parameters

Blood was collected by cardiac puncture in microcentrifuges tubes containing heparin sodium. Blood samples were analyzed for routine hematological parameters. Blood cell counts were done with blood smears (Banerjee *et al*., 1979; Walz *et al*., 1971).

Biochemical Parameters

Biochemical parameters were performed in plasma and serum. Serum Gluatmic Oxaloacetic Transaminase (SGOT), Serum Gluatmic Pyruvic Transaminase activities (SGPT), serum levels of alkaline phosphatase (ALP), Blood Urea Nitrogen (BUN), plasma protein and plasma sugar levels were estimated. All parameters were estimated using Merck semi auto analyzer (Dax-72 autoanalyzer, Bayer Diagnostics; Sysmx-NE-8000 autoanalyzer) using standard kits (Merck India Ltd., Baroda, Gujrat India) (Kalender *et al*., 2009; Bohmer *et al*., 2009).

Histological Examination

At the end of treatment animals were sacrificed and various organs like liver, kidney, lungs and gonads were collected for histological examinations. After fixing the tissues in 10% formalaldehyde the tissues were dehydrated and paraffin blocks were made. Then sectioning was done using Leica microtome and sections of 5-7 µ. Routine histopathology was performed using H and E staining (Tikoo *et al*., 2008).

Statistical Analysis

Results are shown as Mean±SD. Significance of difference between groups was evaluated by using ANOVA. If ANOVA shows significant differences, post hoc analysis was performed with Dunnet test. $p<0.05$ was considered as statistically significant. All analysis were performed with the help of Sigma stat 7.0 software (Tikoo *et al*., 2008).

RESULTS

The results of current study showed no adverse changes in physical parameters throughout the dosing period in rat or mice. No significant changes were observed in the mean body weight of the animals in Sulbactomax treated groups as compared to age matched vehicle treated control group at the end of treatment duration (Table 1, 2). No Mortality was evident from the experimental results in mice as well as rat in Sulbactomax treated groups at any of the dose levels. The food and water intake of all the three groups were comparable to control group without having significant alteration in body weight and growth rate. The site of injection was observed in all animals and no signs of local damage/tissue necrosis were observed at site of injection. The blood was analyzed for cell counts and there were no significant changes were observed in Red Blood Cell (RBC), Total Leukocyte Counts (TLC) and platelet counts in all the treated groups as compared to respective control groups (Table 3, 4). The Haemoglobin level in Sulbactomax treated group in both sexes of rat and mice had not shown any deviation any dose level as compared to control (Table 3, 4). There were no significant alterations seen in total protein level in all the treated groups as compared to control group (Table 5, 6). No significant increase was observed at even highest dose used in this work on liver function tests serum glucose, SGPT and SGOT activities, in all the Sulbactomax treated groups as compared to respective control group. Moreover BUN and

Table 1: Effect of sub acute dose of Sulbactomax on hemogram in male rat

		Sulbactomax (Ceftriaxone-Sulbactam)		
		10	50	150
Parameters	Control	(mg kg^{-1})		
HB (g%)	13.30±0.664	13.01±0.84	14.02±0.93	13.97±0.91
RBC	7.10±0.99	7.22±0.54	7.62±0.97	7.57±0.90
TLC	4260.00±800.18	4370.00±766.42	4513.66±918.01	4433.33±782.0
Platelets	36500.00±10899.549	358600.00±10581.232	366500.00±9813.760	379200.00±0958.8601
ESR (mm h^{-1})	11.01±0.99	11.01±1.59	10.90±1.50	10.90±2.50
Poly (%)	57.66±5.8	59.83±6.55	59.00±6.48	55.67±6.95
Lympho (%)	31.67±4.42	37.20±6.95	34.50±7.674	32.80±8.6
Monocytes (%)	2.2±0.88	2.80±0.95	2.80±0.88	1.93±1.66
Eosinophils (%)	2.11±0.99	1.70±0.55	2.00±0.35	2.18±0.88

Values expressed as Mean±SD, n = 6

Table 2: Effect of sub acute dose of Sulbactomax on biochemical parameter in male rat

		Sulbactomax (Ceftriaxone-Sulbactam)		
		10	50	150
Parameters	Control	(mg kg^{-1})		
Glucose (mg dL^{-1})	88.33±15.77	81.66±12.66	93.90±11.5	95.35±10.48
BUN (mg dL^{-1})	29.93±8.97	25.12±6.73	21.42±7.85	26.33±7.68
Creatinine (mg dL^{-1})	0.89±0.19	0.75±0.54	0.92±0.77	0.81±0.58
TG (mg dL^{-1})	111.0±20.11	104.88±17.19	117.43±16.33	98.50±12.50
SGOT (IU L^{-1})	38.30±6.47	30.67±9.066	31.33±8.15	38.88±9.50
SGPT (IU L^{-1})	33.33±7.52	31.41±11.63	32.16±8.66	34.33±9.55
ALP (IU L^{-1})	110.33±15.66	97.55±12.66	103.33±17.33	106.06±17.53
Na (mEq L^{-1})	138.50±8.43	139.17±5.57	131.87±6.90	130.67±9.80
K (mEq L^{-1})	4.94±0.91	4.20±1.04	3.98±1.11	3.97±0.96
Cl (mEq L^{-1})	99.99±5.60	102.3±8.67	97.87±7.50	93.75±6.96
BIL (mg dL^{-1})	0.87±0.28	0.73±0.069	0.71±0.22	0.87±0.19
Cholesterol (mg dL^{-1})	90.90±11.494	99.80±15.33	94.19±5.92	90.67±13.33
Protein (g dL^{-1})	4.65±0.31	4.71±0.90	4.77±0.68	4.88±0.85

Values expressed as Mean±SD, n = 6

Alkaline phosphatase levels were also found comparable to respective control group at all dose levels of treated group in rat and mice (Table 5, 6).

Histological examination were conducted and there were no significant treatment related histopathological changes were observed in organs of all the treated groups. No damage was observed in the brain section of treated animals as compared to control animals. There was no mortality found till the completion of study.

Table 3: Effect of sub acute dose of Sulbactomax on hemogram in female rat

		Sulbactomax (Ceftriaxone-Sulbactam)		
		10	50	150
Parameters	Control	(mg kg^{-1})		
HB (g%)	11.03±1.84	10.83±0.93	11.50±1.56	10.83±0.65
RBC	5.75±0.84	5.83±0.98	5.77±0.96	5.68±0.80
TLC	3980.833±288.86	3658.333±374.50	3663.333±221.10	3900.667±413.12
Platelets	315500.0±18639.549	311400.0±12591.232	312400.0±2914.760	313800.0±17658.863
ESR (mm h^{-1})	10.99±2.48	11.88±1.69	11.53±2.12	11.50±1.90
Poly (%)	56.87±4.99	51.33±3.63	58.33±6.77	57.55±6.33
Lympho (%)	37.50±3.928	35.65±7.60	37.06±6.33	33.55±8.73
Eosinophils (%)	1.54±0.97	1.60±0.79	1.68±0.84	1.55±0.48
Monocytes (%)	1.97±0.85	1.88±0.76	1.78±0.85	1.81±0.76

Values expressed as Mean±SD, n = 6

Table 4: Effect of sub acute dose of Sulbactomax on biochemical parameter in female rat

		Sulbactomax (Ceftriaxone-Sulbactam)		
		10	50	150
Parameters	Control	(mg kg^{-1})		
GLUCOSE (mg dL^{-1})	92.33±18.79	95.90±14.40	91.65±19.50	101.85±12.88
BUN (mg dL^{-1})	30.84±5.52	25.93±7.15	27.67±5.58	27.81±6.65
Creatinine (mg dL^{-1})	0.83±0.27	0.90±0.28	0.87±0.18	0.85±0.23
TG (mg dL^{-1})	99.00±18.77	109.15±13.80	101.74±10.15	98.60±14.60
SGOT (IU L^{-1})	30.55±7.90	31.40±7.18	31.7±10.60	36.07±7.01
SGPT (IU L^{-1})	33.33±8.66	33.05±9.66	31.40±6.65	31.91±3.55
Na (mEq L^{-1})	138.60±8.53	137.71±4.50	142.33±6.55	135.18±6.90
K (mEq L^{-1})	4.15±0.91	4.53±0.80	4.00±0.98	3.80±0.75
ALP (IU L^{-1})	115.55±13.574	112.50±11.27	99.70±19.2	95.55±21.2
Cl (mEq L^{-1})	94.37±8.31	96.87±9.60	94.71±9.85	99.67±5.9
BIL (mg dL^{-1})	0.80±0.18	0.78±0.22	0.83±0.21	0.81±0.16
Cholesterol (mg dL^{-1})	103.45±9.8	99.9±10.65	91.77±13.47	99.40±13.90
PROTEIN (g dL^{-1})	4.70±0.98	4.79±0.86	4.90±0.73	4.80±0.76

Values expressed as Mean±SD, n = 6

Table 5: Effect of sub acute dose of Sulbactomax on hemogram in male mice

		Sulbactomax (Ceftriaxone-Sulbactam)		
		10	50	150
Parameters	Control	(mg kg^{-1})		
HB (g%)	11.05±0.940	10.68±1.80	11.07±0.99	10.9±0.98
RBC	5.85±0.91	5.70±0.54	5.90±0.75	5.68±0.44
TLC	4880.50±550.040	4550.00±732.23	4590.15±680.18	4840.33±690.92
Platelets	352128.22±5552.600	345700.333±7100.543	354612.18±4200.788	35880.90±4315.500
ESR (mm h^{-1})	10.55±3.10	10.14±1.33	11.00±1.78	10.90±1.80
Poly (%)	66.03±8.45	64.99±5.54	67.43±7.33	64.91±6.52
Lympho (%)	33.33±2.85	32.90±2.97	32.40±2.70	32.44±3.75
Monocytes (%)	1.40±0.55	1.88±0.55	1.33±0.90	1.55±0.85
Eosinophils (%)	1.60±0.50	1.30±0.40	1.20±0.50	1.50±0.24

Values expressed as Mean±SD, n = 6

Table 6: Effect of sub acute dose of Sulbactomax on hemogram in female mice

		Sulbactomax (Ceftriaxone-Sulbactam)		
		10	50	150
Parameters	Control	(mg kg^{-1})		
HB (g%)	10.10±0.81	10.87±0.64	10.17±0.74	11.94±0.77
RBC ($X10^6$ mm^{-3})	6.44±0.41	6.90±0.44	5.88±0.95	6.1±0.53
TLC ($X10^3$ mm^{-3})	5545.50±750.40	5308.88±860.33	5125.55±620.33	5223.28±390.22
Platelets ($X10^5$ mm^{-3})	329250.33± 18250.190	318900.000± 19690.33	317400.500± 21970.563	333679.33± 20710.80
ESR (mm h^{-1})	11.64±1.22	10.90±1.76	11.38±1.95	10.44±1.84
Poly (%)	63.71±3.56	63.48±6.83	63.73±7.11	65.00±4.53
Lympho (%)	34.50±2.87	32.18±5.99	34.91±6.24	33.54±7.077
Monocytes (%)	1.50±0.54	1.94±0.43	1.71±0.51	1.37±0.41
Eosinophils (%)	1.64±0.89	1.45±0.52	1.30±0.30	1.42±0.32

Values expressed as Mean±SD, n = 6

DISCUSSION

The study described here aimed to establish the safety profile of Sulbactomax in rodents. Resistance to third- and fourth-generation cephalosporins has become a major concern worldwide (Li *et al*., 1994; Caron *et al*., 1990). Even more alarming is the emergence of carbapenem resistance; the carbapenems are often considered to be a 'drug of choice' and are increasingly used in empirical therapy for various bacterial infections (Miro *et al*., 1995; Kikuchi *et al*., 2002). The combination of Sulbactam and Ceftriaxone sodium is active against all the organisms showing sensitive to Ceftriaxone. In addition, it demonstrates synergistic activity (reduction in minimum inhibitory concentrations for the combination versus those of each component) in a variety of organisms (Levin, 2002; Bhattacharjee *et al*., 2008; Betrosian *et al*., 2008). Strong efficacy of Ceftriaxone-Sulbactam against wide range of bacteria has been established (Shrivastava *et al*., 2009).

This study was designed to investigate possible toxic outcomes of this promising therapeutic combination. Various physical changes were studied in the treated as well as vehicle treated animal. The IV administration of Sulbactomax had not caused any local injury and inflammatory responses at site of injection in the treated groups. Increase in body weights and growth of treated animals (rat and mice) of either sex were of similar pattern as in age matched control groups. Published evidences also suggest no Ceftriaxone treatment-related changes in organs in any species (Nechifor *et al*., 1992), however no previous reports are available on toxicity profile of ceftriaxone sulbactam combination.

Blood was evaluated for hematological toxicity of FDC. Hemogram was estimated and results showed no deleterious effect on blood cell count, haemoglobin and other related parameters (1, 3, 5 and 6). The biochemical changes were also studies for possible toxic manifestations on kidney and liver function. There were no significant changes in serum alkaline phosphatase, SGOT and SGPT activities in Sulbactomax treated groups of either sex as compared to the respective control group (Table 2, 4, 7 and 8). This confirms the safety profile of Sulbactomax (FDC) for injection in hepatic related aspects.

Sulbactomax is eliminated through renal excretion (Foulds *et al*., 1983; Ripa *et al*., 1990; Bradley *et al*., 1992), thus it was mandatory to estimate effects of FDC on kidney functions. Biochemical parameters related to kidney function were evaluated and no significant differences were observed in blood urea nitrogen (BUN), creatinine, glucose and proteins with respect to control (Table 2, 4, 7 and 8). Previous reports also suggested, no dose-imiting

Table 7: Effect of sub acute dose of Sulbactomax on biochemical parameter in male mice

		Sulbactomax (Ceftriaxone-Sulbactam)		
		10	50	150
Parameters	Control	(mg kg^{-1})		
GLUCOSE (mg dL^{-1})	97.85±21.61	94.00±20.96	90.75±22.56	89.44±17.8
BUN (mg dL^{-1})	31.28±6.8	26.79±7.44	26.95±8.8	29.73±6.80
Creatinine (mg dL^{-1})	0.73±0.079	0.59±0.097	0.75±0.085	0.71±0.088
TG (mg dL^{-1})	180.00±27.76	159.38±29.05	171.16±19.80	182.31±16.4
SGOT (IU L^{-1})	30.57±6.33	31.44±5.53	28.67±6.66	26.05±5.63
SGPT (IU L^{-1})	33.78±9.61	41.20±6.61	38.50±7.81	39.66±8.44
Na (mEq L^{-1})	140.22±4.99	136.66±6.55	139.60±6.57	138.90±4.65
K (mEq L^{-1})	4.23±0.94	3.97±0.669	4.10±0.460	4.15±0.38
ALP (IU L^{-1})	102.17±11.8	97.66±14.22	110.22±21.9	95.67±27.1
Cl (mEq L^{-1})	99.99±8.51	95.80±4.06	93.55±8.22	103.00±8.31
BIL (mg dL^{-1})	0.76±0.29	0.79±0.18	0.74±0.30	0.82±0.19
Cholesterol (mg dL^{-1})	160.26±21.11	163.20±25.76	171.73±24.03	165.53±21.64
Protein (g dL^{-1})	4.92±0.4	5.16±0.42	4.97±0.54	5.22±0.80

Values expressed as Mean±SD, n = 6

Table 8: Effect of sub acute dose of Sulbactomax on biochemical parameter in female mice

		Sulbactomax (Ceftriaxone-Sulbactam)		
		10	50	150
Parameters	Control	(mg kg^{-1})		
GLUCOSE (mg dL^{-1})	92.58±16.54	99.66±17.54	116.78±21.80	93.35±15.8
BUN (mg dL^{-1})	26.33±5.45	28.44±6.58	28.03±13.4	27.65±7.78
CREATININE (mg dL^{-1})	0.80±0.25	0.75±0.19	0.68±0.12	0.71±0.14
TG (mg dL^{-1})	192.50±19.33	185.44±20.44	191.5±13.75	176.45±13.65
SGOT (IU L^{-1})	37.54±8.33	31.73±6.99	34.20±5.90	38.12±6.5
SGPT (IU L^{-1})	29.61±6.3	24.65±7.80	31.20±6.5	28.63±6.45
ALP (IU L^{-1})	114.33±11.66	101.0±19.52	119.0±14.66	99.65±16.55
K (mEq L^{-1})	3.97±0.64	3.71±0.59	3.88±0.66	3.9±0.53
Na (mEq L^{-1})	140.60±12.52	133.81±10.11	137.9±9.3	134.54±7.91
Cl (mEq L^{-1})	94.60±6.8	96.80±6.8	101.1±9.6	98.96±8.31
BIL (mg dL^{-1})	0.84±0.060	0.71±0.080	0.91±0.09	0.83±0.07
CHOLESTEROL (mg dL^{-1})	193.13±27.94	212.55±33.20	190.52±16.55	195.99±21.95
PROTEIN (g dL^{-1})	4.97±0.96	5.05±0.57	4.93±0.87	5.13±0.81

values expressed as Mean±SD, n = 6

toxicity observed in the trial of Ceftriaxone. Both drugs were found to be safe, well tolerated and associated with improvement in the inflammatory symptoms (Bradley *et al*., 1992; Nechifor *et al*., 1992). Similar outcome has been observed in current study with FDC Sulbactomax treatment, however similar studies were not carried out in past for assessment of toxicity profile of fixed dose combination Sulbactomax.

The above mentioned findings are well supported by histopathological outcomes and no signs of toxicity were seen in any of organ in histopathological analysis at all doses of sulbactomax. Thus histopathological studies provides additional strength to the safety data of other physiological, biochemical and heamatological parameters of Sulbactomax treatment. Our data suggest that combination of Sulbactomax is safe at even high dose that is multiple fold of dose intended to be used for human treatment as no clinically relevant alterations of any of the physiological and biochemical parameters were observed in this study. It can be concluded that in preclinical settings (subacute dosing in rat and mice) for 28 days, a parenteral therapy consisting of Sulbactomax, Ceftriaxone together with Sulbactam (a beta lactamase inhibitor), as fixed dose combination offers no obvious toxicity at any dose level.

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