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Acute Oral Toxicity of A Novel Combined Antibiotic (Cefatrizine/Clavulanic Acid) in Rats

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ABSTRACT *: The acute toxicity study of combined antibiotic (Cefatrizine/Clavulanic Acid), a formulation consisting of cafatrizine and clavulanic acid in a ratio of 2 : 1, was evaluated in rats. The antibiotic was orally administered with single dose in dose levels up to 5 g/kg (0, 1.25, 2.5, 5 g/kg). Treatment-related effects were limited to soft stool excretion and caecal dilatation, but histologically no morphological changes could be detected in caecum. In hematology, serum-chemistry parameters and histopathology, no drug-related changes were found. The results of the present study indicate that cefatrizine/clavulanic acid has a low toxic potential and the oral LD_{50} values exceed 5 g/kg in rats*

Key Words *: Combined antibiotic (Cefatrizine/Clavulanic acid), Toxicity, Rat*

I. INTRODUCTION

Expression of β-lactamase is the major mechanism of bacterial resistance to β-lactam antibiotics such as penicillin and cephalosporins. And it is generally accepted that β-lactamase producing bacteria are on increase due to the abuse and misuse of antibiotics and bacterial adaptation, which can be overcome by either developing new β-lactamase-stable antibiotics or developing β-lactamase inhibitors (Coleman, 1995; Payne *et al.*, 1994). Nowadays combination therapy is clearly accepted part of clinical practice and rationally designed combination such as Augmentin™, amoxycillin/clavulanic acid, are well accepted (Cuffini *et al.*, 1996). In case of amoxycillin, an antibiotic with a favorable pharmacological profile, is combined with clavulanic acid, a potent inhibitor of the β-lactamases which are responsible for the majority of resistance to amoxycillin. Recently a new combination antibiotic of cefatrizine/clavulanic acid was developed by Dong-A Pharmaceutical company. Cefatrizine is an orally absorbed cephalosporin with a broad antibacterial spectrum and a high chemotherapeutic potential (Santella and Tanrisever, 1985). The antibacterial effect and bioavailability of cefatrizine and clavulanic acid, given orally as a 2 : 1 formulation, two parts cefatrizine free acid equivalent to one part clavulanic acid has been studied in laboratory animals and experimental infection models (Kim *et al.*, 1998a, b). And the favorable pharmacokinetic profiles of cefatrizine and clavulanic acid and antibacterial efficacy were obtained in preclinical studies. In the literature, oral LD_{50} values of both cefatrizine and clavulanic acid in rodents exceed 5 g/kg. In the present study the acute toxicity of combined antibiotic was investigated in rats.

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II. MATERIALS AND METHODS

1. Test material

The test material, the combined 2 : 1 formulation of cefatrizine and clavulanic acid supplied by Research Laboratory of Dong-A Pharmaceutical company, is a slightly yellowish white crystalline powder. The test substance was stored at room temperature in the dark before use. For oral administration, test material was suspended in 0.5% carboxymethylcellulose (CMC).

2. Test system

All experimental procedures were performed in conformity with *Guide for the Care and Use of Laboratory Animals*. Five-week old Sprague-Dawley (CD) rats of both sexes were purchased from Charles River Japan (Kanagawa). After arrival, the animals were acclimated for one week and used at the age of six weeks. Immediately before dosing, male and female rats weighed 185~194 g and 136~144 g, respectively. The animal room was air-conditioned at 23±3°C with a constant 12 hr (7 : 00~19 : 00) light/dark cycle. The animals were housed five per standard suspended metal cage and allowed free access to a commercial pellet diet (Jeil Feed Co., Korea) and UV-sterilized tap water.

In the preliminary test conducted in 3 male and 3 female rats, no deaths occurred at a dose of 5 g/kg. In this study, therfore, three doses of combined formulation were adopted at 0 (vehicle control), 1.25, 2.5, and 5 g/kg. In each group, five male and five female rats were used. The test material suspended in 0.5% CMC was administered orally using a ball-tipped needle. The dosing volume was 10 ml/kg and calculated for individual animals based on the body weight before administration. The animals were frequently observed for clinical signs of toxicity or mortality on the dosing day and at least twice daily from days 1 through 7. Body weight was measured in all animals immediately before dosing and on days 3 and 7.

All survivors at the end of the observation period underwent laparotomy under ether anesthesia, and blood samples were taken from the abdominal aorta. Part of blood sample was used for hematological tests after the prevention of clotting with EDTA-dipotassium. In the rats from control and high dose (5 g/kg) groups, serum was separated from remaining blood sample, followed by biochemical tests. The animals were killed by bleeding after the blood sampling.

Hematological examination was conducted using an automatic blood cell counter (Minos-Vet) for RBC (erythrocytes), PCV (hematocrit), hemoglobin, WBC (leukocytes) and thrombocytes. Serum biochemistry parameters were determined using an automatic analyzer (Spectrum, Abbott) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total Creatinine (CRE), alkaline phosphatase (ALP) and urea nitrogen (UN).

After sacrificing the animals, autopsy was performed for macroscopic observation of the organs and tissues. Tissue samples of vital organs from all the animals in control and high dose groups as well as from macroscopic lesion were removed and fixed in 10% neutral buffered formalin solution, then processed by routine techniques for paraffin embedding. After sectioning, each specimen was stained with hematoxylin and eosin and examined with light microscope (BH-2, Olympus) by a veterinary pathologist blinded to the study.

Body weight was expressed as mean±standard error of the mean. Data were analyzed statistically using an analysis of variance (ANOVA) procedure and Duncan's multiple range test. For the determination of intergroup difference in the incidence of clinical signs and necropsy findings, R×C Chi square test and Fisher's exact test were performed. P values of ≤ 0.05 were considered statistically significant.

III. RESULTS

No deaths occurred at any dose level during the observation period. There were no discernible clinical signs immediately after oral administration in any treatment group. From day 2, however, soft stool was found in some animals from treatment groups. This sign persisted throughout the observation period although the stool consistency increased in some rats from day 6. All the rats from middle (2.5 g/kg) and high (5.0 g/kg) dose showed soft

Table 1. Incidence of clinical signs and macrosopic necropsy findings in rats orally administered with combined antibiotic (cefatrizine/clavulanic acid)

Signs and necropsy findings	Vehicle		Cefatrizine/clavulanic acid (g/kg)					
	-		1.25		2.5		5.0	
	M	F	M	F	M	F	M	F
Soft stool	0/5	0/5	1/5	2/5	5/5*	5/5*	5/5*	5/5*
Caecal enlargement	0/5	0/5	2/5	2/5	5/5*	5/5*	5/5*	5/5*

M; male, F; female.
*; Significantly different from the control group (α=0.05).

Table 2. Body weight (grams) changes in rats orally administered with combined antibiotic (cefatrizine/clavulanic acid)

Groups	Drug	Dose (g/kg)	Sex	Days after administration		
				Day 0	Day 3	Day 7
1	Vehicle	-	male	190.4±6.1	218.8±10.9	254.8±12.1
			female	141.2±3.6	157.0±4.7	174.6±6.2
2	Cefatrizine/ clavulanic acid	1.25	male	189.4±5.6	214.4±7.7	246.2±10.9
			female	141.2±7.6	161.2±9.6	173.0±11.7
3		2.5	male	191.2±3.8	219.6±6.8	254.6±5.8
			female	139.2±3.0	156.2±4.7	170.0±4.1
4		5.0	male	191.2±4.7	217.2±8.3	251.4±10.4
			female	139.8±5.1	154.2±5.2	164.2±3.2

Each value represents mean±S.D.

Table 3. Hematological parameters in rats orally administered with combined antibiotic (cefatrizine/clavulanic acid)

Sex	Parameters	Vehicle	Cefatrizine/clavulanic acid (g/kg)		
		-	1.25	2.5	5.0
Male	WBC (10^3/mm^3)	6.6±1.0	7.0±1.5	6.7±1.9	7.6±1.3
	RBC (10^6/mm^3)	6.5±0.2	6.1±0.6	6.3±0.7	6.2±0.6
	HB (g/dl)	16.1±0.3	14.9±1.6	14.8±1.5	15.1±1.0
	HT (%)	34.9±1.0	33.1±2.8	33.8±3.7	33.5±2.6
	MCV (μ^3)	53.4±0.9	54.4±1.3	52.8±1.1	54.0±2.2
	MCH (pg)	24.6±0.7	24.5±0.8	23.5±0.8	24.2±1.0
	MCHC (g/dl)	46.0±0.8	45.0±1.1	44.5±1.1	44.9±0.6
	PLT (10^3/mm^3)	701±37	673±41	668±32	672±97
Female	WBC (10^3/mm^3)	5.5±1.8	5.0±0.7	5.0±1.4	5.4±1.0
	RBC (10^6/mm^3)	6.3±0.6	6.4±0.3	6.2±0.3	6.2±0.3
	HB (g/dl)	12.8±3.9	14.9±0.4	14.6±0.8	14.6±0.3
	HT (%)	28.8±7.9	32.6±0.8	32.8±1.0	32.3±0.9
	MCV (μ^3)	52.2±1.9	51.6±1.7	51.8±1.5	51.8±1.1
	MCH (pg)	23.6±1.2	23.3±0.9	23.6±0.8	23.3±0.8
	MCHC (g/dl)	45.3±1.0	45.2±0.6	45.4±0.9	45.0±1.0
	PLT (10^3/mm^3)	670±69	700±31	694±33	698±37

Each value represents mean±S.D.

stool at least once during the observation period (Table 1). There was no inter-group difference in body weight throughout the study period (Table 2).

As depicted in Table III and Table IV, hematological and serum-biochemical analyses showed no abnormal treatment-related changes in any parameters.

Necropsy was performed on day 7. Macroscopic observation revealed cecal dilatation in cefatrizine/clavulanic acid treated rats. Two out of five animals in both sex from low (1.25 g/kg) dose and all the animals from middle and high dose groups showed enlargement of the caecum filled with soft stool (Table 1). Except cecal enlargement, there were no treatment-related macroscopic abnormalities in vital organs in the rats from control and treated groups.

All tissues from control and high-dose groups, including the caecum that showed enlargement in macroscopic findings, revealed no treatment-related histopathological abnormalities (Table V).

Table 4. Serum biochemical parameters in rats treated with combined antibiotic (cefatrizine/clavulanic acid)

Sex	Parameters	Vehicle	Cefatrizine/ clavulanic acid
		-	5.0 g/kg
Male	Total protein (g/dl)	5.85±0.09	5.98±0.20
	Phosphorus (mg/dl)	10.79±1.18	11.50±1.64
	Creatinine (mg/dl)	0.58±0.13	0.64±0.11
	Albumin (g/dl)	4.67±0.09	4.64±0.25
	Calcium (mg/dl)	11.39±0.42	11.53±0.55
	ALT (IU/L)	44.95±4.12	51.10±4.90
	AST (IU/L)	110.96±24.12	112.89±21.86
	BUN (mg/dl)	23.61±2.56	24.73±3.80
	ALKP (IU/L)	454.74±37.75	470.15±41.87
	Triglyceride (mg/dl)	258.30±21.11	255.05±16.80
	Cholesterol (mg/dl)	104.91±4.46	106.28±10.31
Female	Total protein (g/dl)	5.67±0.37	5.63±0.36
	Phosphorus (mg/dl)	10.29±0.83	9.27±0.37
	Creatinine (mg/dl)	0.47±0.15	0.41±0.04
	Albumin (g/dl)	4.74±0.26	4.80±0.25
	Calcium (mg/dl)	11.17±0.98	10.74±0.68
	ALT (IU/L)	41.70±5.53	45.67±5.00
	AST (IU/L)	103.01±19.12	117.32±25.03
	BUN (mg/dl)	20.05±2.67	20.28±3.12
	ALKP (IU/L)	374.76±36.20	397.80±65.81
	Triglyceride (mg/dl)	215.21±23.40	188.78±28.35
	Cholesterol (mg/dl)	109.73±17.93	104.44±7.45

Each value represents mean±S.D.

Table 5. Microscopic observations in rats orally treated with combined antibiotic (cefatrizine/clavulanic acid)

Organs and Findings	Groups	Cefatrizine/clavulanic acid (g/kg)							
		Vehicle		1.25		2.5		5.0	
	Sex	M	F	M	F	M	F	M	F
Brain		(5)	(5)	-		-		(5)	(5)
Within normal limits		5	5	-		-		5	5
Lung		(5)	(5)	-		-		(5)	(5)
Alveolar congestion		1	0	-		-		1	1
Peribronchial in-		0	0	-		-		0	0
Heart		(5)	(5)	-		-		(5)	(5)
Within normal limits		5	5	-		-		5	5
Thymus		(5)	(5)	-		-		(5)	(5)
Mild congestion		1	1	-		-		0	0
Liver		(5)	(5)	-		-		(5)	(5)
Vacuolar degeneration		0	0	-		-		0	0
Slight congestion		1	0	-		-		0	0
Small intestine (duodenum, jejunum, ilieum)		(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Within normal limits		5	5	5	5	5	5	5	5
Large intestine (cecum, colon)		(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Within normal limits		5	5	5	5	5	5	5	5
Spleen		(5)	(5)	-		-		(5)	(5)
Brown pigment		1	0	-		-		0	0
Kidney		(5)	(5)	-		-		(5)	(5)
Interstitial cell infiltration		5	5	-				5	5
Adrenal gland		(5)	(5)	-		-		(5)	(5)
Within normal limits		5	5	-		-		5	5
Testes		(5)	-	-		-		(5)	-
Within normal limits		5	-	-		-		5	-
Seminal vesicles		(5)	-	-		-		(5)	-
Within normal limits		5	-	-		-		5	-
Prostate gland		(5)	-	-		-		(5)	-
Within normal limits		5	-	-		-		5	-
Ovaries		-	(5)	-		-		-	(5)
Within normal limits		-	5	-		-		-	5

The Number in parenthesis is the number of animals examined.
-; Not examined.

IV. DISCUSSION

The β-lactam family of antibiotics is the largest single group of antibiotics used in clinical practice. However, since the most of bacteria produced the β-lactam degrading enzymes known as β-lactamases, widespread emergence of bacterial resistance is a remarkable problem. So, developing of new β-lactamase-stable antibiotics or new classes of β-lactamase inhibitors or combination therapy are of interest for clinical reasons (Coleman, 1995; Martin and Jones, 1995).

Cefatrizine is an orally absorbed cephalosporin that can be administered either parenterally or orally, and clavulanic acid is a potent inhibitor of the β-lactamases which are responsible for the majority of bacterial resistance. In the present study the acute toxicity of combined antibiotic (cefatrizine/clavulanic acid, 2 : 1) was investigated in rats.

The present acute toxicity study of combined antibiotic (cefatrizine/clavulanic acid), orally administered to rats, revealed no significant changes in any of the measured parameters. However, mild to moderate gastrointestinal changes including soft stool, diarrhea and caecal dilatation were observed in combined antibiotic-treated rats with a tendency of dose-relation. These changes, however, were not accompanied by histological alterations in the caecal wall, and no increase in tissue mass occurred (data not shown). Such findings are not unique to cefatrizine/clavulanic acid : caecal enlagement is a common response of rats to treating high doses of a variety of antibiotics such as beta-lactams (Hashimoto, *et al.*, 1996; Welles, 1972), cephalosporins (Hanasono, *et al.*, 1979; Kadota, *et al.*, 1990), macrolides(Ikezaki *et al.*, 1995) and combined antibiotic (Hayashi *et al.*, 1994). Matsuzaki *et al.* (1976a, b) also showed that cefatrizine affected the changes in intestinal microflora and therefore soft stool, diarrhea and caecal enlargement occured in acute and subacute toxicity studies in rats. The mechanism underlying diarrhea and caecal enlargement has not completely been elucidated, but it is now widely accepted that the phenamenon may be induced by a change of intestinal microflora (Garilla, 1975; Leegwater, 1974). Hashimoto *et al.*, (1996) showed that the beta-lactam antibiotics decreased intestinal bacteria, increased the caecal weight, and a pronounced enlargement of the caecum is a general feature in rats. The results of this study described above suggested that cefatrizine/clavulanic acid also brought soft stools and caecal enlargement, but the enlargement is considered a common findings and/or a physiological responses attributed to the antibiotics and it is never accompanied by histopathological changes in the intestine, it is considered to be of only minor, if any, toxicological importance. In other parameters including hematology, serum-chemistry

and histopathology, no drug-related changes were found. These findings are comparable to the results of a previous study showing that cefatrizine had no treatment-related changes in rats except for the changes in intestinal microflora, soft stool, diarrhea and caecal enlargement (Matsuzaki *et al.*, 1976a, b).

In summary, a single administration of combined antibiotic (cefatrizine/clavulanic acid) to rats at dose levels ranging 1.25 g/kg to 5 g/kg resulted in no toxicologically significant changes except marginal effects on gastrointestinal tract; soft stools and caecal enlargement. Based on these findings, it is concluded that the combined antibiotic, cefatrizine/clavulanic acid, has a low toxic potential and the oral LD_{50} values exceed 5 g/kg in rats.

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