

Single and Repeated-Dose Toxicity of TR-701 (DA-7218) in Rodents

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D. KIM, H. CHUNG, S. CHOI, W. IM, J. RHEE
Dong-A Pharm., Yongin-Si, Republic of Korea.

Contact Information:

Donghwan Kim
Dong-A Pharm. Co., Ltd. Research Lab.
47-5 Sanggal-Dong Kiheung-Gu
Yongin-Si
Kyunggi-Do 449-905 Korea
e-mail: dhkim@donga.co.kr

Abstract

Background: TR-701 (DA-7218), an oxazolidinone antibiotic, has demonstrated excellent efficacy in animal models of Gram-positive bacterial infections when administered both orally (PO) and intravenously (IV). For the safety evaluations of TR-701, single and repeat-dose toxicity studies in rodents were performed.

Methods: TR-701 was tested at single doses with both sexes of SD rats and ICR mice up to 250 mg/kg (IV) and 2000 mg/kg (PO). Mortality, clinical signs, and body weight were observed for 2 weeks. Also, body weight, food consumption, ophthalmology, urinalysis, hematology, serum biochemistry, necropsy, organ weight, histopathology, and immunotoxicity were evaluated in rats after oral administration of TR-701 for 4-weeks, in dose ranges from 10 to 100 mg/kg.

Results: No mortality was observed in mice after any oral dose. Mortality was observed in the single IV 250 mg/kg dose in 2/5 male and 1/5 female mice. In the single dose rat toxicity study, mortality was observed at the 2000 mg/kg oral dose (2/5 female), and in the single IV 250 mg/kg (3/5 males and 3/5 females). Toxicokinetics revealed the dose-dependent increase in the systemic exposure for both sexes, but higher levels were observed in females on both day 1 and day 28.

Conclusions: In oral single dose toxicity studies, the minimum LD (lethal dose) of TR-701 was determined to be over 2000 mg/kg for both sexes of mice, 2000 mg/kg for female rats and over 2000 mg/kg for male rats. In intravenous single dose toxicity studies, the LD₅₀ was determined to be 256 mg/kg in male mice, 274 mg/kg in female mice and 244 mg/kg in both sexes of rats. The NOAEL of the repeat-dose toxicity in rat was established to be 30 mg/kg for males and 10 mg/kg for females.

Repeat oral toxicity study in SD rats

In order to investigate the repeated oral dose toxicity of TR-701 (DA-7218), the test article was administered orally to 4 groups of SD rats at dose level of 0, 10, 30 and 100 mg/kg/day for 4 consecutive weeks (15 animals/sex/group, except for 10 animals/sex/group in the 10 mg/kg/day group). Ten animals per sex in all groups were sacrificed at the end of the 4-week dosing period. For toxicokinetics evaluation, the test article was also administered to an additional 3 groups (11 animals/sex/group) at dose levels of 10, 30 and 100 mg/kg/day for 4 consecutive weeks. Study parameters included the clinical signs, body weight changes, food consumption, ophthalmologic examinations, urinalysis, hematology, serum biochemistry measurements, necropsy, organ weight measurement, histopathological examinations, immunotoxicity evaluation and toxicokinetics evaluation.

Results

□ Acute rodent toxicity

- There was no mortality in the oral administration, but 2 males and 1 female died in the intravenous administration from high-dose animal (250 mg/kg) in mice.
- Two females from high-dose animals (2000 mg/kg) died in the oral administration, and 3 males and 3 females died in the intravenous administration from high-dose animal (250 mg/kg) in rats.

□ 4-weeks repeat toxicity study of rats

- All females from high dose animals (100 mg/kg) died with several clinical signs, and histopathological changes including hematopoietic and lymphatic organ, liver, etc. (Table 1)
- Also, decreases of body weight (Figure 1, 2) and food consumptions were observed from high-dose animals (100 mg/kg) in females.
- Hematology revealed the decreased WBC, reticulocyte, and neutrophil counts, and also showed the increased MCV, MCH, and lymphocyte from mid-dose animals (30 mg/kg) in females. (Table 2)
- TK revealed the dose-dependent increase of systemic exposure for both sexes, but it was higher in female on both day 1 and day 28. (Table 3)

Table 1. Microscopic findings of females in rat 4 week repeat toxicity study

Organ	Dose (mg/kg/day)			
	0	10	30	100
No. of examination	10	10	10	0 (11)+
Liver				
Hepatocellular hypertrophy	0	-	0	0 (4)
Thymus				
Atrophy	0	0	0	0 (11)
Spleen				
Atrophy	0	0	0	0 (8)
Stomach				
Erosion	0	-	0	0 (3)
Duodenum				
Mucosal inflammation/atrophy	0	0	0	0 (11)
Ulcer	0	0	0	0 (1)
Erosion	0	0	0	0 (7)
Cecum				
Atrophy	0	-	0	0 (5)
Mesenteric lymph node				
Atrophy	0	-	0	0 (8)
Femur				
Bone marrow atrophy	0	0	5*	0 (11)
Sternum				
Bone marrow atrophy	0	0	6*	0 (11)

*: P < 0.05, +: The number in the parenthesis is numbers of animals found dead and sacrificed moribund.

Table 2. Hematological findings of males and females in rat 4 week repeat toxicity study

Dose (mg/kg)	WBC (x10 ³ /ul)	MCV (fL)	MCH (pg)	RET (%)	NEU (%)	LYM (%)
Male						
0	10.95	59	19.4	2.2	11.2	83.4
10	9.18	59.1	19.5	2.1	10.7	84.7
30	10.3	59.3	19.6	2.4	5.6*	89.9*
100	8.57	62.9**	21.0*	2.1	8.0*	86.7
Female						
0	7.77	58.1	19.6	2.2	10.6	83.9
10	7.74	58.6	20.1	2.2	6.7*	87.9*
30	6.01*	59.8*	20.5*	1.2*	5.0*	89.4*
100	(6.04)+	(57.2)	(20.4)	(0.2)	(32.4)	(64.3)

*: p < 0.05, **: p < 0.01, +: The result in the parenthesis is from the sacrificed moribund animals at day 10.

Table 3. Toxicokinetics in rat 4-week repeat toxicity study

Dose (mg/kg)	Male			Female		
	C (µg/mL)	T (hr)	AUC _{0-24h} (ng*hr/mL)	C (µg/mL)	T (hr)	AUC _{0-24h} (ng*hr/mL)
Day 1						
10	5679	0.67	29139	27382	4.67	309566
30	19000	2.67	99288	29371	2.67	320552
100	72479	4.67	740321	72798	6	828473
Day 28						
10	6622	0.83	23330	10458	0.42	54141
30	23598	1.17	81485	24106	2.25	219644
100	55130	3.33	402853	33128	2.33	289954

Fig. 1. Mean body weight changes of males in rat 4-week repeat toxicity study

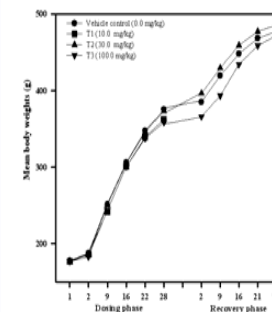
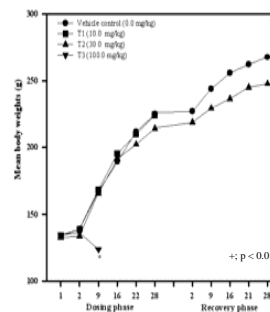


Fig. 2. Mean body weight changes of females in rat 4-week repeat toxicity study



Introduction

Oxazolidinones are a novel structural class of antibiotics that display a unique mechanism of action. This class of compounds inhibits protein synthesis by preventing binding of *N*-formylmethionyl-tRNA (fm-tRNA) to the 70S ribosome, thereby inhibiting translation of the initiation step. In particular, binding studies have demonstrated that oxazolidinones interact with the 50S ribosomal subunit and prevent the formation of the fm-tRNA, 50S subunit, and 70S subunit complex (also called the "70S complex") (Zurenko *et al.*, 2001). This mechanism is different from other inhibitors of protein synthesis that specifically target the elongation phase of protein synthesis (*e.g.*, macrolides, streptogramins). TR-701 (DA-7218) is an oral and IV therapeutic agent that is being developed for the treatment of Gram-positive bacterial infections. TR-701 (DA-7218) is the phosphate salt of the active species, TR-700 (DA-7157), and was developed as a result of the poor solubility and absorption of TR-700 (DA-7157). TR-701 (DA-7218) is a prodrug that is rapidly converted to TR-700 (DA-7157) in the plasma of animals and humans. For the purpose of safety evaluations, single and repeat toxicity studies (GLP) in rodents were performed.

Methods

Single intravenous toxicity study in ICR mice and SD rats

To evaluate the acute toxicity of TR-701 (DA-7218) after a single intravenous administration, four groups of 15 male and 15 female ICR mice and SD rats (120 total of each species) respectively. The test item was given once at the doses of 0, 62, 125 and 250 mg/kg. The mortality, clinical signs, body weight gains and gross findings were continually screened until 15 days following the single dose.

Single oral toxicity study in ICR mice and SD rats

To evaluate the acute toxicity of TR-701 (DA-7218) after a single oral administration, 20 male and 20 female animals were randomly grouped into 4, respectively for ICR mice and SD rats. The test item was given once at the doses of 0, 500, 1000 and 2000 mg/kg. The mortality, clinical signs, body weight gains and gross findings were continually screened until 21 days following the single dose.

Conclusions

In intravenous single-dose toxicity studies, the LD₅₀ was 256 mg/kg in male mice, 274 mg/kg in female mice, and the LD₅₀ of rats was 244 mg/kg in both sexes. In oral single toxicity studies, the LD₅₀ of mice was over 2000 mg/kg in both sexes, and the LD₅₀ of rats was 2052 mg/kg for female and LD₅₀ for male was over 2000 mg/kg. The NOAEL of the 4-week oral repeat toxicity in rats were established to be 30 mg/kg for males and 10 mg/kg for females respectively, and the target organs were determined to the hematopoietic and lymphatic organs, such as bone marrow, thymus, spleen, and lymph node.