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Acute Toxicity of CKD-602, a New Anticancer Agent, in Rats

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Abstract – The present study was carried out to investigate the potential acute toxicity of CKD-602 by a single intravenous dose in Sprague-Dawley rats. Ten males and ten females were used in each test groups: a vehicle control, 34.7, 41.7, 50.0, 60.0 and 72.0 mg/kg groups, and were given different single intravenous doses of CKD-602 to the test animals. Mortalities, clinical findings, and body weight changes were monitored for the 14-day period following the administration. At the end of 14-day observation period, all animals were sacrificed and complete gross postmortem examinations were performed. One, 1, 2, 8 and 9 cases of deaths occurred in the male dose groups of 34.7, 41.7, 50.0, 60.0 and 72.0 mg/kg, respectively, and 1, 5 and 9 cases in the female dose groups of 50.0, 60.0 and 72.0 mg/kg, respectively. An increase in the incidence of clinical signs such as alopecia, skin pallor, skin ulcerations, emaciation and change of fecal material was found in the both sexes of all treatment groups. A decrease or suppression in the body weight was also observed in a dose-dependent manner. In autopsy, male and/or female rats of the treatment groups showed treatment-related gross findings such as splenomegaly, atrophy of the testis, epididymis, seminal vesicles, ovary, uterus and thymus which were dose-dependent in incidence and severity. Based on these results, it was concluded that a single intravenous injection of CKD-602 to rats caused significant toxicities in gastrointestinal, hematopoietic, and reproductive systems. The LD_{50} value was 53.8 (95% confidence limit: 48.5~60.6) mg/kg for males and 60.1 (95% confidence limit: 55.3~65.8) mg/kg for females. The LD_{10} value was 39.9 (95% confidence limit: 31.7~44.8) mg/kg for males and 50.3 (95% confidence limit: 40.6~54.8) mg/kg for females.

Keywords □ anticancer agent, CKD-602, camptothecin, acute toxicity, LD_{50} value, rats

INTRODUCTION

Camptothecin (CPT) is a cytotoxic alkaloid extracted from the bark, fruit, and leaves of the Chinese tree *Camptotheca acuminata*. Although some antitumor activity was observed, its development was hampered by poor solubility and unpredictable toxicities such as hemorrhagic cystitis, myelosuppression, and diarrhea (Gottlieb *et al.*, 1970; Moertel *et al.*, 1972; Slichenmyer and Rowinsky, 1993; Takimoto *et al.*, 1998; Pizzolato and Saltz, 2003). Since then, extensive efforts to develop structural analogues of CPT were begun with the aim of overcoming the two key limiting factors in development of the parent drug. This resulted in the discovery of a number of CPT analogues such as CPT-11 (irinotecan), topotecan and 9-aminocamptothecin (9-AC) (Bleiberg and Rothenberg, 1996; Dahut *et al.*, 1996; Kolimannsberger *et al.*, 1999). The mechanism of action of CPT derivatives lies in the inhibition of topoisomerase I which is an important nuclear enzyme for various DNA functions including transcription and replication. Because they cause DNA damage, the CPTs are potentially mutagenic and can induce chromosomal aberrations including increased sister chromatid exchanges, gene deletions, and gene rearrangements (Hashimoto *et al.*, 1995). DNA synthesis inhibiting agents and DNA damaging agents are well known to produce toxic side effects on multiple organ systems (Kim *et al.*, 2003a; Kim *et al.*, 2004). The most common adverse effects associated with CPTs are diarrhea and myelosuppression (Pizzolato and Saltz, 2003).

CKD-602 is a new camptothecin derivative antitumor agent with a formula (7-[2-(N-isopropylamino)ethyl]-(20S)-camptothecin) developed by Chong Kun Dang Pharmaceutical Company in Korea (Lee *et al.*, 1998; Kim *et al.*, 1998). Like other camptothecin derivatives, CKD-602 is a potent inhibitor of topoisomerase I, and successfully overcomes the poor water

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solubility and toxicity of the parent drug. Preclinical studies of CKD-602 demonstrated broad antitumor activity against various human tumor cell lines, and the results were equal or superior to those of camptothecin and topotecan, a clinically active antitumor drug (Lee *et al.*, 1998; Lee *et al.*, 2000; Kim *et al.*, 2003b). CKD-602 showed significant anticancer activity against gastric and ovarian cancer.

As a part of safety evaluation studies of the test article, CKD-602, a single intravenous dose toxicity study was performed in Sprague-Dawley rats. The present study was conducted according to the test guidelines from the Korea Food and Drug Administration (KFDA) and Organisation for Economic Cooperation and Development (OECD) guidelines for the testing of chemicals under modern Good Laboratory Practice Regulations.

MATERIALS AND METHODS

Animal husbandry and maintenance

Seventy-two Sprague-Dawley rats of each sex were obtained from the Korea Institute of Toxicology at 4 weeks of age and used after 1 week of quarantine and acclimatization. The animals were housed in a room maintained at a temperature of 23 ± 3°C and a relative humidity of 50 ± 10% with artificial lighting from 08:00 to 20:00 and with 13-18 air changes per hour. Only healthy animals were assigned to the study. The animals were housed two per cage in stainless steel wire mesh cages and were allowed sterilized tap water and commercial rodent chow (Jeil Feed Co, Daejeon, Korea) *ad libitum*. This experiment was conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International), and animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council).

Test article and preparation

CKD-602, a colorless white powder, was chemically synthesized and provided by Chong Kun Dang Pharmaceuticals Co. (Seoul, Korea). The chemical structure of CKD-602 is depicted in Fig. 1. CKD-602 was dissolved in distilled water with D – mannitol 50 mg, tartaric acid 0.06 mg in 1 ml and adjusted to pH 3.5 and was prepared immediately before the treatment and those of lower groups were prepared by stepwise dilution of that of the highest dose group.

Experimental groups

Healthy males and females were randomly assigned to six

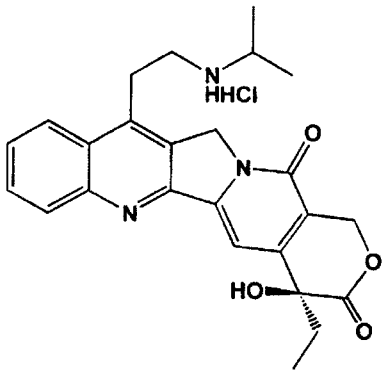

Fig. 1. Chemical structure of CKD-602

experimental groups: five treatment groups receiving 34.7, 41.7, 50.0, 60.0, or 72.0 mg/kg groups and a vehicle control group. Each group consisted of 10 rats of each sex.

Selection of doses

In a preliminary test with 4 males and 4 females per group, 3 males and 4 females at 60 mg/kg, 2 males and 1 male at 44.4 mg/kg, and 1 male at 32.9 mg/kg were found dead. From the results, the LD_{50} values were estimated 45 mg/kg in males and 47 mg/kg in females. Based on the results of the study, doses of 72.0, 60.0, 50.0, 41.7, and 34.7 mg/kg were selected using a scaling factor x1.2. In addition, a vehicle control group was added to determine the effects of vehicle.

Treatment

The test article was injected by 25G needle into a lateral tail vein at the speed of 2 ml/min after skin was disinfected with 70 % alcohol-cotton. The application volume (10 ml/kg) was calculated according to the body weight on the treatment day. The intravenous route is the clinically intended route for the test article.

Mortality and clinical observation

Clinical signs and mortality were checked every hour until 6 hour after dosing and then once a day thereafter up to day 14. Detailed clinical observations were recorded and printed by a Labcat System (Innovative Programming Associates Inc., NJ, USA).

Body weight

Individual body weights of animals were measured shortly before the test article administration and on days 1, 3, 7 and 14 after the treatment thereafter.

Necropsy

On day 14 after the treatment, all animals were euthanized by carbon dioxide overdose and necropsied with special attention to all vital organs and tissues.

Statistical analysis

LD_{50} values were analysed by Probit method using a Labcat System, and LD_{10} values were analyzed using a Statistical Analysis Systems (SAS Institute, Inc., 1997). Body weight values were presented by mean ± S.D.

RESULTS

Mortality and lethal dose

The mortalities for the male and female rats treated with CKD-602 by a single intravenous injection are presented in Table I. The number of dead animals was 1, 1, 2, 8 and 9 cases for the 34.7, 41.7, 50.0, 60.0, and 72.0 mg/kg male groups, respectively, and 1, 5 and 9 cases for the 50.0, 60.0 and 72.0 mg/kg female groups, respectively. Death was observed in the male groups between days three and nine after injection, except one case which occurred one day after injection in the 72.0 mg/kg group and another case which occurred eleven days after injection in the 50 mg/kg group. In the female groups, death was observed between days five and ten, except for one case which occurred right after injection in the 60.0 mg/kg group and another case which occurred on the thirteenth day in the 72.0 mg/kg group. As a result, the LD_{50} value is 53.8 (95% confidence limit: 48.5~60.6) mg/kg in males and 60.1 (95% confidence limit: 55.3~65.8) mg/kg in females. The LD_{10} value is 39.9 (95% confidence limit: 31.7~44.8) mg/kg in males and 50.3 (95% confidence limit: 40.6~54.8) mg/kg in females.

Clinical findings

As shown in Table II, treatment-related clinical signs, including hair loss, paleness of skin, skin ulceration, emaciation, watery stool, soft stool, bloody stool, dark-red discoloration of the injection sites, etc., were observed in all dose groups tested. These signs were increased dose-dependently in incidence and severity. The onset time of the symptoms caused by this test article was three days after the treatment, except for hair loss observed from the first day after the treatment.

Body weight changes

As shown in Table III, reduced or suppressed body weights were found from days 1 through 7 after the treatment in all treatment groups of both sexes. Except for 1 case in the 60.0 mg/kg male group, which was not able to recover its weight until the fourteenth day; all the others were able to recover. All dead rats showed a decrease in the body weight before death.

Gross findings

The results of gross postmortem examinations are shown in Table IV. Enlargement and white colored membrane of the

Table I. Mortality of rats after single intravenous injection of CKD-602

Dose (mg/kg)	0	34.7	41.7	50.0	60.0	72.0
Day 0	0/0[a)]	0/0	0/0	0/0	0/1	0/0
Day1	0/0	0/0	0/0	0/0	0/0	1/0
Day 2	0/0	0/0	0/0	0/0	0/0	0/0
Day 3	0/0	0/0	0/0	0/0	0/0	2/0
Day 4	0/0	1/0	0/0	0/0	1/0	0/0
Day 5	0/0	0/0	0/0	0/0	3/2	3/4
Day 6	0/0	0/0	0/0	0/0	1/0	1/3
Day 7	0/0	0/0	0/0	1/0	2/2	1/0
Day 8	0/0	0/0	1/0	0/0	0/0	0/1
Day 9	0/0	0/0	0/0	0/0	1/0	1/0
Day 10	0/0	0/0	0/0	0/1	0/0	0/0
Day 11	0/0	0/0	0/0	1/0	0/0	0/0
Day 12	0/0	0/0	0/0	0/0	0/0	0/0
Day 13	0/0	0/0	0/0	0/0	0/0	0/1
Day 14	0/0	0/0	0/0	0/0	0/0	0/0
Total	0/0	1/0	1/0	2/1	8/5	9/9

[a)]: No. of male/female rats died

Table II. Clinical findings of rats after single intravenous injection of CKD-602

Dose (mg/kg)	0	34.7	41.7	50.0	60.0	72.0
Emaciation	0/0[a)]	0/0	0/0	0/2	3/6	4/9
Paleness of skin color	0/0	0/1	2/4	4/5	5/4	3/2
Tremors	0/0	0/0	0/0	0/0	0/0	1/0
Decreased respiratory rate	0/0	0/0	0/0	0/1	1/0	0/1
Dyspnea	0/0	0/0	0/0	0/0	0/0	1/0
Decreased locomotor activity	0/0	0/0	0/0	1/1	10/5	8/9
Dark material around eyes	0/0	1/1	0/1	2/0	0/0	2/0
Ptosis	0/0	0/0	0/0	0/0	0/0	2/0
Congestion of eyes	0/0	0/0	0/0	0/0	0/0	1/0
Low body tone	0/0	0/0	1/0	0/0	0/0	0/0
Lying on side	0/0	0/0	0/0	0/0	0/0	1/0
Dark-red discoloration of tail	0/0	0/0	0/0	1/0	0/1	1/7
Hair loss	0/0	10/10	10/10	10/10	8/9	4/6
Paralysis of hind limbs	0/0	0/0	0/0	0/0	0/0	1/0
Soft stool	0/0	2/1	7/4	7/8	4/7	3/2
Bloody stool	0/0	0/0	0/0	1/1	1/3	0/1
Watery stool	0/0	0/1	2/2	6/3	9/7	8/9
Soiled fur	0/0	0/0	0/0	1/0	8/4	5/9
Ulceration	0/0	4/4	4/6	4/2	0/3	1/0
Death	0/0	1/0	1/0	2/1	8/5	9/9

[a)]No. of male/female rats with the clinical signs

Table III. Body weight changes of rats after single intravenous injection of CKD-602

Dose (mg/kg)	0	34.7	41.7	50.0	60.0	72.0
Male						
Day 0	140.7 ± 11.31	142.2 ± 8.56	141.4 ± 13.43	141.9 ± 9.87	140.3 ± 10.86	142.6 ± 10.75
Day 1	145.1 ± 12.02	134.6 ± 6.68	134.7 ± 12.36	134.1 ± 8.58	133.6 ± 9.63	136.4 ± 11.31
Day 4	164.5 ± 9.71	137.2 ± 12.06	134.1 ± 13.11	129.5 ± 6.28	119.0 ± 10.64	121.5 ± 11.24
Day 7	195.3 ± 14.29	166.0 ± 9.94	153.5 ± 19.06	144.9 ± 12.57	123.6 ± 4.94	123.3 ± 25.10
Day 14	255.1 ± 18.35	223.9 ± 10.85	204.6 ± 17.74	195.4 ± 14.00	165.4 ± 52.40	161.3
Female						
Day 0	121.5 ± 6.09	122.3 ± 7.39	121.5 ± 7.41	117.8 ± 6.11	121.0 ± 8.26	118.8 ± 6.99
Day 1	124.9 ± 5.31	118.1 ± 7.13	117.9 ± 6.67	115.8 ± 5.57	115.1 ± 6.34	114.8 ± 7.28
Day 4	137.1 ± 6.42	121.9 ± 8.69	117.7 ± 8.27	113.1 ± 8.90	105.8 ± 4.11	102.8 ± 10.50
Day 7	153.9 ± 8.49	142.1 ± 10.33	135.3 ± 7.55	125.9 ± 12.90	116.5 ± 11.71	104.5 ± 20.36
Day 14	177.5 ± 9.47	171.6 ± 10.58	164.6 ± 10.49	158.6 ± 10.97	144.2 ± 16.65	145.4

Values are means ± SD (g)

spleen and atrophy of the prostates, testes, seminal vesicles, epididymides, ovaries and uterus were the major findings in the live rats. Atrophy of the thymus and spleen and dark-red discoloration of the heart and the gastrointestinal tract were the major findings in the dead rats. These gross findings were increased dose-dependently in incidence and severity.

DISCUSSION

The test article CKD-602 was intravenously given to male and female SD rats to evaluate the acute toxicity of CKD-602 at dose levels of 0, 34.7, 41.7, 50.0, 60.0 and 72.0 mg/kg. Clinical signs, mortality, body weight changes and gross findings were observed for fourteen days following the single injection.

In this test, except for the deaths of two female rats, one from the 60 mg/kg group and the other one from the 72 mg/kg group, all deaths occurred after the third day, suggesting delayed toxicity of the test article. This delayed toxicity has been already reported on the former cancer chemotherapeutic agents (Chatelut *et al.*, 2003). The onset time of the symptoms caused by this

Table IV. Gross findings of rats after single intravenous injection of CKD-602

Dose (mg/kg)		0	34.7	41.7	50.0	60.0	72.0
Heart:	dark-red discoloration	0/0[a)]	0/0	0/0	0/0	8/1	3/1
	dark-red spots	0/0	0/0	0/0	0/1	0/0	0/0
Thymus:	dark-red discoloration	0/0	0/0	1/0	3/0	0/0	0/0
	atrophy	0/0	0/0	0/0	0/1	9/7	7/10
Lung:	dark-red discoloration	0/0	0/0	1/0	1/0	4/1	1/1
	edema	0/0	0/0	0/0	0/1	0/0	0/0
Thoracic cavity:	bloody fluid	0/0	0/0	0/0	1/0	0/0	0/0
Liver:	paleness	0/0	0/0	0/0	1/1	2/5	2/2
	atrophy	0/0	0/0	0/0	0/0	1/0	0/0
	white spots	0/0	0/0	0/0	0/0	0/0	1/0
	adhesion with spleen	0/0	0/0	0/0	0/1	0/0	0/0
Spleen:	enlargement	0/0	9/10	9/7	8/8	1/6	1/1
	white colored membrane	0/0	0/3	1/0	4/1	0/0	0/0
	atrophy	0/0	0/0	0/0	1/1	0/0	1/1
	paleness	0/0	0/0	0/0	0/0	1/0	0/0
	dark-red spots	0/0	0/0	0/0	0/0	1/0	0/0
Kidney:	paleness	0/0	0/0	0/0	1/3	3/4	2/2
Adrenal gland:	dark-red discoloration	0/0	0/0	0/0	1/0	3/0	1/0
	enlargement	0/0	0/0	0/0	0/0	1/0	0/0
	paleness	0/0	0/0	0/0	0/0	0/3	1/1
Stomach:	dark-red discoloration	0/0	0/0	0/0	0/0	5/2	3/8
	dark-red discoloration	0/0	0/0	0/0	1/0	3/0	0/0
Cecum:	gas filled	0/0	1/0	0/0	0/0	0/0	6/0
	dark-red discoloration	0/0	1/0	1/0	2/1	8/4	0/8
Mesentric lymph node:	dark-red discoloration	0/0	0/0	0/0	0/0	1/0	0/0
Urinary bladder:	dark-red discoloration	0/0	0/0	0/0	0/0	1/0	1/0
Prostate:	atrophy	0/-	7/-	8/-	8/-	2/-	0/-
Seminal vesicle:	atrophy	0/-	7/-	8/-	8/-	2/-	0/-
	dark-red discoloration	0/-	0/-	0/-	2/-	0/-	0/-
Testis:	atrophy	0/-	8/-	9/-	8/-	2/-	1/-
	dark-red discoloration	0/-	0/-	0/-	0/-	0/-	1/-
Epididymis:	atrophy	0/-	8/-	9/-	8/-	2/-	0/-
	dark-red discoloration	0/-	0/-	0/-	0/-	1/-	0/-
Ovary:	atrophy	-/0	-/0	-/0	-/0	-/3	-/1
Uterus:	enlargement	-/0	-/1	-/1	-/0	-/4	-/0
	atrophy	-/0	-/0	-/0	-/0	-/0	-/1

[a)]No. of male/female rats with the gross findings

test article, except for hair loss, was three days after the treatment. Hair loss observed from the first day after injection cannot be said to be an effect of the test article but a stress effect of the injection. Significant hair loss could be seen after the third day, which can be safely said to be the effect of the test article. Changes of the fecal material were also found. Variable manifestations of abnormal fecal materials, such as watery stool, soft stool and bloody stool, could be observed after the third day of injection. The changes of the feces remained until 8~12 days and then recovered, but in the high dose groups, they remained until death. Soiled fur was a secondary change due to watery stool. Change of the fecal material is a common finding of camptothecin class chemotherapeutic agents and is thought to be related to the dark red discoloration or spots of the gastrointestinal tract (Takimoto *et al.*, 1998; Pizzolato and Saltz, 2003). Paleness of the skin is considered to be a secondary effect induced by the suppression of hematopoietic organs and the bloody stool was observed in the high dose groups.

The dose-dependent decrease of the body weights with increasing dose indicates that this finding is caused by the administration of CKD-602. Dark-red discoloration of the gastrointestinal tract, heart and other organs observed in the dead

animals shows that the use of this test article over lethal dose induces congestion and/or hemorrhage not only in the gastrointestinal tract but in other organs. Atrophy of the male reproductive organs observed in the live animals treated with CKD-602 suggests that overdose of this test article has toxic effects on the male sex organs just like other chemotherapeutic agents (Kim *et al.*, 1999). Uterine hypertrophy in dead females and atrophy of ovaries and uterus in live females suggest that this article also has adverse effects on the female reproductive organs. Splenomegaly or atrophy of the spleen, atrophy or dark-red discoloration of the thymus, and some dark-red discoloration of the lymph nodes show that this article has an effect on the lymphoid tissue. In the case of the spleen, atrophic change was found in the dead animals, and in the live animals hypertrophic change and white membrane formation were observed, suggesting recovery of the damaged organ.

Camptothecin anticancer agents are new class of cancer chemotherapeutic agents suppressing the topoisomerase I enzyme which rises in high level in solid tumor mass (Pratesi *et al.*, 1995; Pizzolato and Saltz, 2003). Because of the distinctive mechanism of action, however, campthothecin derivatives have various adverse effects on multiple organs containing self-renewing cell populations such as bone marrow, gastrointestinal tract, mucosal membrane, reproductive organs, and hair follicles. The pathologic findings observed in the present study also occurred in those organs with high cell division rate, as is the specific character of cancer chemotherapeutic agents with toxic effects on highly proliferating organs and tissues.

On the basis of the results, intravenous injection of CKD-602 to male and female rats had toxic adverse effects on gastrointestinal, hematopoietic, and reproductive systems. The LD_{50} value was 53.8 (95% confidence limit: 48.5~60.6) mg/kg for males and 60.1 (95% confidence limit: 55.3~65.8) mg/kg for females. The LD_{10} value was 39.9 (95% confidence limit: 31.7~44.8) mg/kg for males and 50.3 (95% confidence limit: 40.6~54.8) mg/kg for females.

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