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개에서 새로운 감토테신계 항암제 CKD-602의 단회투여독성시험

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Single dose toxicity study of CKD-602, a new camptothecin anticancer agent, in Beagle dogs

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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 Beagle dogs. The test chemical was administered intravenously to male and female Beagle dogs at dose levels of 0.3, 0.5, or 2.5 mg/kg. 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. All males and females of the 2.5 mg/kg dose group were found dead between the fourth and seventh day after the injection. Treatment related clinical signs, including vomiting, anorexia, mucous stool, diarrhea, and no stool were observed. Decrease or suppression of body weight was observed in a dose-dependent manner. In autopsy, dark red discoloration of the gastrointestinal tract, atrophy of the thymus, paleness of the spleen, sporadic dark red spots of the lung and petechia of the heart were observed in dead animals of the 2.5 mg/kg dose group. There were no specific adverse effects on males and females of the 0.3 and 0.5 mg/kg dose groups, except for the transient clinical signs such as anorexia, vomiting, and mucus/no stool. On the basis of the results, it was concluded that a single intravenous injection of CKD-602 to Beagle dogs resulted in increased incidence of abnormal clinical signs and death, decreased body weight, and increased incidence of abnormal gross findings. The absolute toxic dose of this chemical was 2.5 mg/kg for both genders. The LD_{50} value was 1.1 mg/kg (95% confidence limit not specified) for both genders. The no-observed-effect level (NOEL) was considered to be below 0.3 mg/kg for both genders.

Key words : Anticancer agent, CKD-602, camptothecin, acute toxicity, LD_{50} value, dogs

Introduction

Camptothecin (CPT) is a cytotoxic alkaloid extracted from the bark, fruit, and leaves of the Chinese tree *Camptotheca acuminata* [18, 19]. Although some antitumor activity was observed, its development was hampered by poor solubility and unpredictable toxicities such as hemorrhagic cystitis, myelosuppression, and

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diarrhea [3, 15, 21, 22]. 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) [1, 2, 12]. 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 [5, 6]. 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 [4]. DNA synthesis inhibiting agents and DNA damaging agents are well known to produce toxic side effects on multiple organ systems [9, 10]. The most common adverse effects associated with CPTs are diarrhea and myelosuppression [19].

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 [11, 13]. Like other camptothecin derivatives, CKD-602 is a potent inhibitor of topoisomerase I, and successfully overcomes the poor water 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 [8, 11, 14]. 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 Beagle dogs. The present study was conducted according to the test guidelines from the Korea Food and Drug Administration [7] and Organisation for Economic Cooperation and Development [16] guidelines for the testing of chemicals under modern Good Laboratory Practice Regulations.

Materials and Methods

Animal husbandry and maintenance

Six Beagle dogs (*Canis familiaris*) of each sexes aged 4 months were purchased from Covance Research Product Inc. (Cumberland, VA, USA). During quarantine (5 weeks), each dog was given a complete physical examination; all health parameters were normal. The animals were housed in a room maintained at a temperature of 23±3ºC and a relative humidity of 50±10% with artificial lighting from 07:00 to 19:00 and with 13~18 air changes per hour. Only healthy animals were assigned to the study. The dogs were housed singly in a stainless wire cage. Food (Japan Oriental Yeast Company, Tokyo, Japan) was restricted to 300 g per day (the remaining food was weighed to measure net food intake/animal) with water *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 chemical

CKD-602, a colorless white powder, was chemically synthesized and provided by Chong Kun Dang pharmaceuticals (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 m*l* and adjusted to pH 3.5 and was prepared immediately before the treatment. 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 three experimental groups of CKD-602 receiving

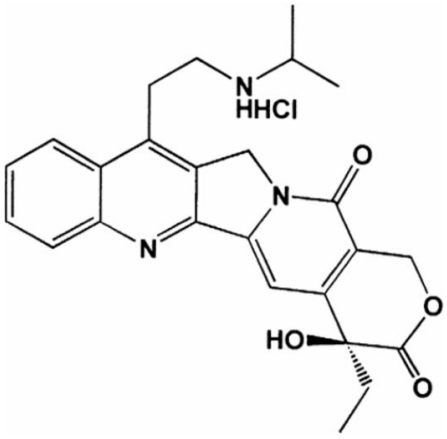

Fig. 1. Chemical structure of CKD-602.

0.3, 0.5 or 2.5 mg/kg. Each group consisted of 2 dogs of each sex. The experimental doses were selected based on the results of a preliminary dose-range finding study.

Treatment

The test article was administered to dogs as a single i.v. bolus injection via a cephalic vein at the speed of 2 m*l*/min. The application volume (10 m*l*/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 Labcat System (Innovative Programming Associates Inc., NJ, USA), respectively.

Body weight

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

Necropsy

On day 14 after the treatment, all surviving animals were anesthetized by the injection of pentotal sodium and then sacrificed by exsanguination from the axillary artery. Complete gross postmortem examinations were performed on all terminated and dead animals.

Statistical analysis

LD_{50} values were analysed by Probit method using a Labcat System (Innovative Programming Associates Inc., NJ, USA). Body weight values were presented by means±S.D.

Results

Mortality and LD_{50} value

The mortalities for the male and female dogs treated with CKD-602 by a single intravenous injection are presented in Table 1. All males and females of the 2.5 mg/kg dose group were found dead between the fourth and the seventh day - 1 case each on the sixth and seventh day for males and 1 case each on the fourth and the seventh day for females. In the 0.3 and 0.5 mg/kg dose groups of both genders, no treatment related deaths were observed. Accordingly, LD_{50} value of both genders is 1.1 mg/kg (95% confidence limit not specified) and the maximum non-lethal dose is 0.5 mg/kg in this test.

Table 1. Mortality of dogs after single intravenous injection of CKD-602 (n=2)

Dose(mg/kg)	0.3	0.5	2.5
Days 0~3	0/0[a)]	0/0	0/0
Day 4	0/0	0/0	0/1
Day 5	0/0	0/0	0/0
Day 6	0/0	0/0	1/0
Day 7	0/0	0/0	1/1
Days 8~14	0/0	0/0	0/0
Total	0/0	0/0	2/2

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

Clinical findings

As shown by the data in Table 2, major treatment related general symptoms were vomiting, mucous stool, diarrhea and anorexia. In the male 2.5 mg/kg dose group, vomiting occurred after 2 hours of injection until the fourth day; anorexia occurred between the second day and the sixth day; no stool occurred between the third day and the fourth day; and mucous stool occurred between the fifth day and sixth day. In the male 0.5 mg/kg dose group, vomiting occurred after 2 hours of injection until the third day and anorexia occurred on the third day. In the male 0.3 mg/kg dose group, vomiting occurred after 2 hours of injection until the third day and anorexia occurred between the second and third day. In the female 2.5 mg/kg dose group, vomiting occurred after 2 hours of injection until the fourth day; decreased locomotor activity occurred between the fifth and the sixth hour of injection; anorexia and mucous stool occurred between the first and the sixth day; diarrhea occurred on the second day; no stool occurred between the third day and the fourth day. In the female 0.5 mg/kg dose group, anorexia occurred between the first and the fourth day, mucous stool on the second day, and vomiting and no stool on the third day. In the female 0.3 mg/kg dose group, vomiting occurred after five hours of injection until the first day, anorexia occurred between the first and the third day, and mucous stool occurred on the

Table 2. Clinical findings of dogs after single intravenous injection of CKD-602 (n=2)

Dose	(mg/kg)	Findings	Days after treatment														
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Male	0.3	Appears normal	2[a)]	1	1	0	2	2	2	2	2	2	2	2	2	2	2
		Anorexia	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
		Vomiting	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	0.5	Appears normal	1	2	2	0	2	2	2	2	2	2	2	2	2	2	2
		Anorexia	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
		Vomiting	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	2.5	Appears normal	0	1	0	0	0	0	0	0	-[b)]	-	-	-	-	-	-
		Anorexia	0	0	2	2	2	2	1	0	-	-	-	-	-	-	-
		Vomiting	2	1	2	2	1	0	0	0	-	-	-	-	-	-	-
		No stool	0	0	0	0	2	0	0	0	-	-	-	-	-	-	-
		Mucus stool	0	0	0	0	0	2	1	0	-	-	-	-	-	-	-
		Death	0	0	0	0	0	0	1	1	-	-	-	-	-	-	-
Female	0.3	Appears normal	1	0	0	0	2	1	2	2	2	2	2	2	2	2	2
		Anorexia	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0
		Vomiting	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		Mucus stool	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	0.5	Appears normal	2	1	0	0	1	2	2	2	2	2	2	2	2	2	2
		Anorexia	0	1	2	2	1	0	0	0	0	0	0	0	0	0	0
		Vomiting	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
		No stool	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
		Mucus stool	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	2.5	Appears normal	0	1	0	0	0	0	0	0	-	-	-	-	-	-	-
		Anorexia	0	1	2	2	1	1	1	0	-	-	-	-	-	-	-
		Decreased locomotor activity	2	0	0	0	0	0	0	0	-	-	-	-	-	-	-
		Vomiting	2	0	1	2	1	0	0	0	-	-	-	-	-	-	-
		Diarrhea	0	0	1	0	0	0	0	0	-	-	-	-	-	-	-
		No stool	0	0	0	2	1	0	0	0	-	-	-	-	-	-	-
		Mucus stool	0	1	0	0	0	0	1	0	-	-	-	-	-	-	-
		Death	0	0	0	0	1	0	0	1	-	-	-	-	-	-	-

[a)] : Number of animals with the clinical signs
[b)] : Data unavailable

fifth day. Except for the treatment related deaths of the 2.5 mg/kg dose group of both genders, which occurred between the fourth and the seventh day after injection, the general symptoms disappeared after the sixth day of treatment in all the remaining animals.

Body weight changes

As shown in Table 3, in males, decrease in body weight was observed in one case from both the 0.5 and 2.5 mg/kg dose groups on the first day, and one case each on the first day and the seventh day from the 0.3 mg/kg dose group. In females, decrease in body weight was observed on the first day in 2 cases from the 2.5 mg/kg dose group and in one case of the 0.5 mg/kg dose group. Also, from the 0.3 mg/kg group, one case showed decrease in body weight between the first day and the seventh day, compared with the starting weight.

Gross findings

The results of gross postmortem examinations are shown in Table 4. In the males of the 2.5 mg/kg dose group, dark red discoloration of the intestine, dark red discoloration of the stomach and atrophy of the thymus were observed in two cases, and multifocal dark red spots on the lung and paleness of the spleen were observed in one case. In the females of the 2.5 mg/

Table 3. Body weight (g) changes of dogs after single intravenous injection of CKD-602

Dose (mg/kg)		Animal No.	Days after treatment				
			0	1	7	14	Gain
Male	0.3	1	7297	7395	7287	7548	251
		2	7589	7568	7653	7911	322
		Mean±SD	7442±208.6	7482±122.3	7470±258.8	7730±256.7	287±50.2
	0.5	3	8093	8000	8214	8641	548
		4	7188	7325	7374	7769	581
		Mean±SD	7641±639.9	7663±477.3	7794±594.0	8205±616.6	565±23.3
	2.5	5	7402	7290	-[a)]	-	-112
		6	7728	7749	-	-	21
		Mean±SD	7570±237.6	7520±324.6	-	-	-45.5±94.0
Female	0.3	7	7263	7155	7201	7565	302
		8	6671	6701	6806	7550	879
		Mean±SD	6967±418.6	6928±321.0	7004±279.3	7559±8.4	591±408.0
	0.5	9	6862	6835	6955	7291	429
		10	7493	7671	7812	8140	647
		Mean±SD	7178±446.2	7253±591.1	7384±606.0	7716±600.3	538±154.2
	2.5	11	7252	6705	-	-	-547
		12	7702	7359	-	-	-343
		Mean±SD	7477±318.2	7032±462.4	-	-	-445±144.3

[a)]: Data unavailable

Table 4. Gross findings of dogs after single intravenous injection of CKD-602

Dose (mg/kg)		0.3	0.5	2.5
Heart:	petechia	0/0[a)]	0/0	0/1
Thymus:	atrophy	0/0	0/0	2/2
Lung:	multifocal dark-red foci	0/0	0/0	1/1
Spleen:	paleness	0/0	0/0	1/1
Stomach:	dark-red discoloration	0/0	0/0	2/2
Small intestine:	dark-red discoloration	0/0	0/0	2/2

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

kg dose group, dark-red discoloration of the stomach and intestine and atrophy of the thymus were observed in two cases, multifocal dark-red spots on the lung and paleness of the spleen were observed in one case, and petechia of the heart was observed in one case. There were no gross pathologic findings in both males and females of the 0.3 and 0.5 mg/kg dose groups.

Discussion

The test article CKD-602 was intravenously given to male and female Beagle dogs to evaluate the acute toxicity of CKD-602 at dose levels of 0.3, 0.5, or 2.5 mg/kg. Clinical signs, mortality, body weight changes, and gross findings were observed for fourteen days following the single injection.

Treatment related deaths occurred from the fourth day after injection in both sexes of the 2.5 mg/kg dose group. Treatment related clinical signs, as evidenced by dose-dependent increases in the incidence and severity of vomiting, anorexia, no stool and mucous stool, were observed at all dose levels. These clinical signs observed in this study are most common adverse effects of camptothecin class chemotherapeutic agents [19, 22]. The clinical signs observed sporadically in both sexes of the 0.3 and 0.5 mg/kg dose groups were not observed from the sixth day after treatment.

A decrease or suppression in body weight was observed in all test groups from the first to the seventh day after treatment. With the disappearance of general symptoms, this change was recovered after the seventh day. An increase in the incidence and severity of abnormal gross findings observed in the treatment groups indicates that it was caused by the injection of CKD-602 because this finding exhibited a dose-response relationship and was consistent with the abnormal clinical signs. Abnormal gross findings such as dark-red discoloration of the gastrointestinal tract and atrophy of thymus are thought to be similar to the changes

mentioned in a report on acute toxicity of hycamtin, a similar compound to the test article [23]. The target organs were said to be bone marrow, lymphoid tissues, alimentary tract and ovaries. Recently, it was reported that major adverse effects observed in general toxicity studies of irinotecan were hematologic and gastrointestinal toxicities [17].

Camptothecin anticancer agents are new class of cancer chemotherapeutic agents suppressing the topoisomerase I enzyme which rises in high level in solid tumor mass [19, 20]. 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 necropsy findings observed in the present study mainly occurred in gastrointestinal tract having a very high cell proliferation rate.

On the basis of the results, it was concluded that a single intravenous injection of CKD-602 to Beagle dogs resulted in increased incidence of abnormal clinical signs and death, decreased body weight, and increased incidence of abnormal gross findings. The absolute toxic dose of this chemical was 2.5 mg/kg for both genders. The LD_{50} value was 1.1 mg/kg (95% confidence limit not specified) for both genders. The no-observed-effect level (NOEL) was considered to be below 0.3 mg/kg for both genders. This dose corresponds to the 15 times of the anticipated human clinical dose of CKD-602, i.e. 0.02 mg/kg/day.

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