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Comparison of Single-Dose Toxicity by Intravenous Infusion or Bolus Injection with CKD-602, a Camptothecin Anticancer Agent in Rats (I): Toxic Effects with regard to Mortality and Clinical Signs

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ABSTRACT. The toxicity of CKD-602 was investigated at doses of 0, 3, 9, and 27 mg/kg in rats, by administering the same total dose over 24-hr continuous infusion or bolus injection. CKD-602 treatment caused gastrointestinal symptoms such as diarrhea, soft stool, and soiled perineal region. It also decreased body weight at doses of 9 and 27 mg/kg in a dose-dependant manner. At 3 mg/kg, clinical signs and body weight decrease were more severe in the infusion group than in the bolus group. In the bolus group, mortalities were 0/8, 0/8, 1/8, and 3/8 at 0, 3, 9, and 27 mg/kg, respectively, whereas those were 0/8, 1/8, 8/8, and 8/8 in the infusion group. LD_{50} values were 36.25 mg/kg for bolus and 3.50 mg/kg for infusion, respectively. This finding indicates that the toxic potency of CKD-602 by continuous infusion is about 10 times higher than by bolus injection. Our findings suggest that the toxic effects of CKD-602 are dependant upon the duration of intravenous administration.

Keywords: CKD-602, Anticancer, Infusion, Bolus, Toxicity, Rat.

INTRODUCTION

Camptothecin (CPT) is a plant antitumor alkaloid isolated from *Camptotheca acuminata* (Wall *et al.*, 1966). Although CPT was previously found to have antitumor activity, its development was halted due to poor solubility and unpredictable toxicity, which included hemorrhagic enterocolitis and myelosuppression in clinical trials (Gottlieb *et al.*, 1972; Moertel *et al.*, 1972; Slichenmyer and Rowinsky, 1993; Takimoto *et al.*, 1998; Pizzolato and Saltz, 2003). Since then, extensive efforts have been made to develop structural analogues of CPT with the aim of overcoming the these two key limiting factors 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 mode of mechanism of CPT derivatives involves the inhibition of topoisomerase I, which is an important nuclear enzyme for various DNA functions, including transcription and replication (Hertzberg *et al.*, 1989). However, the CPTs have been reported to commonly cause some adverse effects, such as diarrhea and myelosuppression (Pizzolato and Saltz, 2003).

CKD-602 is a new camptothecin derivative and an anticancer agent, and was developed by the Chong Kun Dang Pharmaceutical Company (Lee *et al.*, 1998). CKD-602 (mol. wt. 470.0 Da and melting point of 240~242°C) is pale yellow solid with the chemical name (7-[2-(N-isopropylamino)ethyl]-(20S)-camptothecin). It is highly water-soluble and has potent anticancer activity against gastric and ovarian cancer. A preclinical pharmacologic evaluation of CKD-602 demonstrated that it has broad anticancer activity against various human tumor cell lines, which is equal or superior to those of other camptothecin analogs (Lee *et al.*, 1998).

A number of studies showed improved efficacy for compounds (e.g., IGF-1, heparin, growth hormone, interferon gamma etc.) when administered by continuous

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infusion rather than by bolus injection (Tomas *et al.*, 1996; Edelman and Karnovsky 1994; Gargosky *et al.*, 1994; Flynn *et al.*, 1993). Similarly, in the case of CPTs, preclinical studies suggest that protracted administration schedules produce greater antitumor effects than bolus administration (Thompson *et al.*, 1998; Jung and Zamboni 2001). However, it is possible that the continuous infusion of CPTs also cause more severe adverse effects than bolus injection. However, safety assessment of CPTs has not yet clarified in an animal model with respect to continuous intravenous infusion. To evaluate the safety of CKD-602, we utilized an effective rat model of continuous intravenous infusion (Kim *et al.*, 1996) and compared the toxicity of CKD-602 by bolus injection and 24-hr continuous infusion.

MATERIALS AND METHODS

Test item

CKD-602 (purity ≥ 98.3%) was supplied from Chong Kun Dang Pharmaceutical Co. (Seoul, Korea). The dosing solution was prepared by dissolving the test item at the maximum dosage with 100 mg of D-mannitol and 0.12 mg of tartaric acid in 2 ml of distilled water (vehicle), and adjusted to pH 3.5. This solution was serially diluted to prepare the test item for administration. The dosing solutions were prepared immediately before the treatment.

Animal husbandry and Treatment group

Sixty-four Sprague-Dawley male rats were obtained from Orient Co. (Seoul, Korea) at 9 weeks of age. 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 to 18 air changes per hour. Thirty-two rats with or without catheterization surgery were assigned to the infusion (I) or the bolus (B) groups, which were subdivided into four dose subgroups (VC, T1, T2, T3); 0 (VC-I or VC-B), 3 mg/kg (T1-I or T1-B), 9 mg/kg (T2-I or T2-B), and 27 mg/kg (T3-I or T3-B). Animals were given the same total dose by either 24-hr continuous infusion or bolus injection.

The animals were allowed sterilized tap water and commercial rodent chow (Jeil Feed Co, Daejeon, Korea) *ad libitum*, and only healthy animals were used in the study. This experiment was conducted in facilities approved by the Association for the 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).

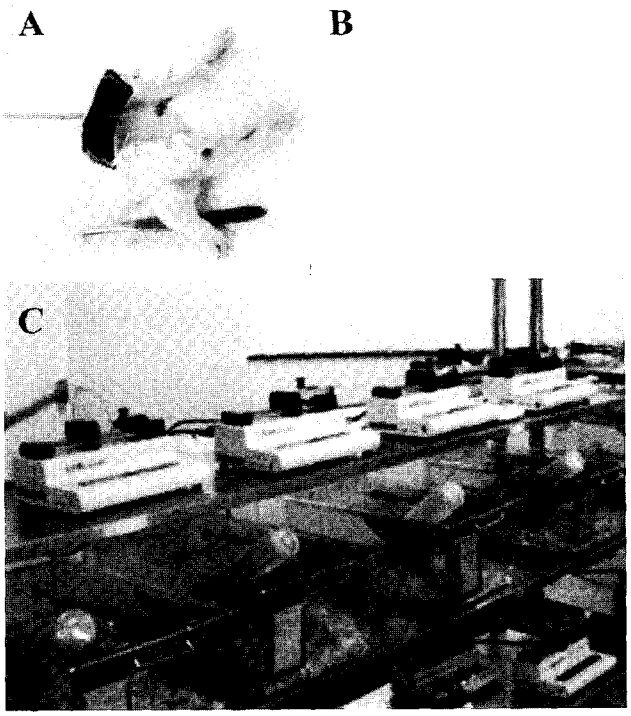

Fig. 1. Intravenous infusion system. A: Catheter is passed through a subcutaneous tunnel and exteriorized at the back of the neck. B: Rat Jacket for a hydraulic swivel-tethering system. C: Intravenous infusion using infusion pump.

Surgical procedures for intravenous 24 hr-infusion using infusion pump

The details of the surgical procedure used in the present study have been previously reported (Kim *et al.*, 1996). In brief, a polyethylene catheter (PE-50) was inserted into the jugular vein under anesthetic pentobarbital sodium in the thirty-two rats. The catheter was then passed through a subcutaneous tunnel and exteriorized at the back of the neck (Fig. 1). It was connected to a hydraulic swivel-tethering system. Rats were allowed to recover for approximately 48 hr after surgery. The catheterized rats were administered with test article for 24 hr using an infusion pump with 20 ml/kg/day (KB Scientific, Model 100, USA).

Clinical signs and mortality

Animals were observed daily for clinical signs of toxicity, morbidity, and mortality throughout the study. Detailed clinical observations were recorded using Labcat program (Innovative Programming Associates Inc., New Jersey, USA).

Body weight changes

Body weights were measured shortly before the test item treatment and on days 1, 3, 7, and 14 after the

treatment therefore.

Necropsy

On the 14th day after treatment, complete gross post-mortem examinations were performed on all terminated animals; euthanized by carbon dioxide overdose.

Statistical analysis

Statistical analyses were performed by comparing the different dose subgroups with Statistical Analysis Systems software (SAS/STAT Version 8.1, Cary, NC, USA). Variance homogeneity was examined using the Bartlett test. If the Bartlett test indicated no significant deviations from variance homogeneity, the ANOVA multiple comparison test (Dunnett test) was conducted to determine which pairs of group comparison were significantly different. The level of significance was taken as $P<0.05$ or 0.01. Survival rates were analyzed using Log-Rank test (JMP version 4, SAS Institute Inc. Cary, NC, USA).

RESULT

Mortality and survival rate

The mortalities of the bolus and infusion groups treated with CKD-602 are presented in Table 1. Mortalities were dose-related and these were much higher in the infusion groups than in the bolus groups. In the bolus group, the mortalities were 0/8, 0/8, 1/8, and 3/8, at 0, 3, 9, and 27 mg/kg, respectively, whereas those were 0/8, 1/8, 8/8, and 8/8 in the infusion group. The LD_{50} values were 36.25 mg/kg in the bolus group and 3.50 mg/kg in the infusion group, respectively. These data indicate that the toxic potency of CKD-602 by continuous infusion is about 10 times greater than by bolus injection.

As shown in Fig. 2, all dead rats in the 3, 9, and 27 mg/kg dose subgroups were found before seventh day. At 3 mg/kg dose, seven rats survived to sacrifice in the infusion group, whereas all eight rats survived in the bolus group. At 9 and 27 mg/kg, no rats survived to terminal sacrifice in the infusion group, while seven or five rats survived, respectively, in the bolus group. These demonstrate that infusion was associated with a significantly shorter survival rate ($p<0.001$).

Table 1. Mortality and LD_{50} of rats treated with CKD-602 by bolus injection or 24 hr infusion

Days after dosing	0 mg/kg		3 mg/kg		9 mg/kg		27 mg/kg	
	B	I	B	I	B	I	B	I
D0	0/8*	0/8	0/8	0/8	0/8	0/8	0/8	0/8
D1	0/8	0/8	0/8	0/8	0/8	4/8	1/8	5/8
D2	0/8	0/8	0/8	0/8	0/8	0/8	1/8	3/8
D3	0/8	0/8	0/8	0/8	0/8	1/8	0/8	0/8
D4-D5	0/8	0/8	0/8	1/8	0/8	1/8	0/8	0/8
D6-D7	0/8	0/8	0/8	0/8	1/8	2/8	1/8	0/8
D8-D14	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Total	0/8	0/8	0/8	1/8	1/8	8/8	3/8	8/8
LD_{50}	Bolus injection : 36.25 mg/kg, 24-hr infusion : 3.50 mg/kg							

*No. of animals dead/No. of animals.

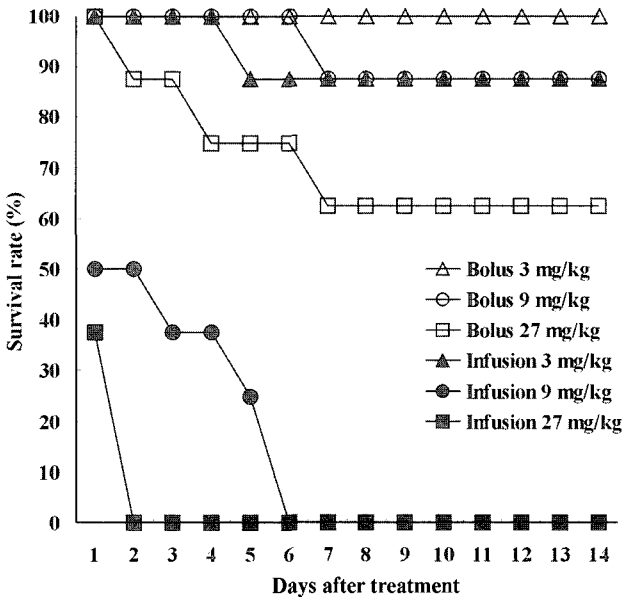

Fig. 2. Survival rate. All dead rats of 3, 9, and 27 mg/kg dose groups were found between the first and seventh day and thereafter the other survived to the terminal day. ***$p<$ 0.001 (Statistical comparison by Log-Rank test was carried out between bolus group and infusion group).

Clinical signs

The clinical signs observed in the bolus and infusion groups are presented in Table 2. Major treatment-related clinical signs were diarrhea, soft stool, soiled perineal region, and decreased locomotor activity; the gastrointestinal symptoms were observed about 3 days after treatment. At 3 mg/kg, the infusion group showed 5 cases of diarrhea, 2 of soft stool, 4 of soiled perineal region, and 1 case of decreased locomotor activity, whereas the bolus group showed no gastrointestinal symptoms. At 9 mg/kg, the bolus group showed 4 cases of diarrhea, 3 of soft stool, 2 of soiled perineal region, whereas the infusion group showed 3 cases of diarrhea, 1 of soft stool, 3 of soiled perineal region, and 5 of decrease of locomotion activity. At 27 mg/kg, the infusion group showed 2 cases of decreased locomotion activity, whereas the bolus group showed 4 cases of diarrhea, 2 of soft stool, 4 of soiled perineal region, and 2 of decreased locomotor activity.

Table 2. Incidence of clinical signs of rats treated with CKD-602 by bolus injection or 24-hr infusion

Clinical signs observed	Onset of symptoms	0 mg/kg		3 mg/kg		9 mg/kg		27 mg/kg	
		B	I	B	I	B	I	B	I
Diarrhea	D3	0/8*	0/8	0/8	5/8	4/8	3/3	4/6	-
Soft stool	D3	0/8	0/8	0/8	2/8	3/8	1/3	2/6	-
Soiled perineal region	D3	0/8	0/8	0/8	4/8	2/8	3/3	4/6	-
Decreased Locomotor activity	D1	0/8	0/8	0/8	1/8	0/8	5/8	2/8	2/8

*No. of animals with the sign/No. of animals.

Table 3. Body weights of rats treated with CKD-602 by bolus injection or 24-hr infusion

	0 mg/kg		3 mg/kg		9 mg/kg		27 mg/kg	
	B	I	B	I	B	I	B	I
Day 0	293.6 ± 23.2[1)]	290.8 ± 22.6	299.1 ± 14.8	296.8 ± 11.1	293.1 ± 19.6	299.4 ± 13.7	299.1 ± 13.1	299.8 ± 12.0
Day 1	293.3 ± 14.0	281.6 ± 23.1	283.7 ± 15.8	282.3 ± 8.8	275.1 ± 19.0	286.2 ± 11.7	281.4 ± 13.0	293.2 ± 6.0
Day 3	306.1 ± 8.8	295.4 ± 25.1	297.2 ± 14.4	257.0 ± 15.3***	263.4 ± 19.0	238.2 ± 5.3	253.7 ± 8.8	-
Day 7	320.1 ± 16.9	312.1 ± 34.4	318.5 ± 14.8	264.3 ± 34.9**	279.5 ± 45.3	-	260.1 ± 31.0	-
Day 14	310.0 ± 12.3	306.4 ± 19.3	310.0 ± 12.1	274.6 ± 31.3*	297.0 ± 10.7	-	269.0 ± 16.9	-

[1)]Represents mean ± SD (bolus, n=5~8; infusion, n=7~8).
*$p<0.05$, **$p<0.01$, ***$p<0.001$ (Statistical comparison was carried out between bolus group and infusion group).

Body weight

As shown in Table 1, CKD-602 by either bolus injection or 24-hr infusion produced dose-related effects on body weight. The bolus group showed significant body weight decreases at 9 and 27 mg/kg and body weight was decreased in a dose-dependant manner (Table 3). At 3 mg/kg, body weights on days 3 and 7 post-treatment were more decreased in the infusion group than in the bolus group, and the infusion administrations of more than 9 mg/kg caused death. It seems that decreases in body weights on days 3, 7 and 14 after treatment is associated with the onset of symptoms of day 1 or day 3 after treatment.

Gross findings

As shown in Table 4, on day 14 post-treatment, the 3 mg/kg infusion subgroup showed one case each of fibrous capsule of the spleen, atrophy of the thymus, enlargement of the submandibular lymph node, and atrophy of the testes, but the bolus group showed no gross findings. The 9 mg/kg bolus subgroup showed one case of adhesion to diaphragm in liver, one case of fibrous capsule in the spleen, and two cases of atrophy in the thymus, and the 27 mg/kg subgroup showed one case of fibrous capsule in the spleen, one case of pale kidney, three cases of atrophy thymus, and two cases of atrophy of testes.

DISCUSSION

In the present study, we utilized a rat model of continuous intravenous infusion. Continuous intravenous infusion has several advantages, which include high bioavailability, straightforward assessment of pharmacokinetic parameters under a steady-state condition. This method is available for the rat, dog, mouse and rabbit, and has also been applied to primates (Evans and Kerry, 2000). Continuous intravenous infusion had been

Table 4. Gross findings of rats treated with CKD-602 by bolus injection or 24 hr-infusion

Organs	Gross findings	0 mg/kg		3 mg/kg		9 mg/kg		27 mg/kg	
		B	I	B	I	B	I	B	I
Liver	Adhesion to diaphragm	0/8*	0/8	0/8	0/7	1/7	-	0/5	-
Spleen	Fibrous capsule	0/8	0/8	0/8	1/7	1/7	-	1/5	-
Kidney	Pale	0/8	0/8	0/8	0/7	0/7	-	1/5	-
Thymus	Atrophy	0/8	0/8	0/8	1/7	2/7	-	3/5	-
Submandibular Lymph node	Enlargement	0/8	0/8	0/8	1/7	0/7	-	0/5	-
Testis	Atrophy	0/8	0/8	0/8	1/7	0/7	-	2/5	-

*No. of animals with the sign/No. of animals examined.

utilized to administer many compounds, such as, IGF-1, heparin, growth hormone, and interferon gamma (Tomas *et al.*, 1996; Edelman and Karnovsky 1994; Gargosky *et al.*, 1994; Flynn *et al.*, 1993). Thus, we compared the acute toxicities of CKD-602 by bolus injection and 24-hr continuous infusion upon administering the same total doses.

Treatment-related clinical signs, as evidenced by a dose-dependent increase in the incidence and severity of diarrhea, soft stool, soiled perineal region, and decreased locomotor activity, were observed at doses of 3, 9, and 27 mg/kg (Table 2). This finding indicates that CKD-602 induces gastrointestinal toxicity in rats. These gastrointestinal symptoms are consistent with reports on the adverse effect of camptothecins, in which CPTs-treated dogs showed intestinal toxicity that included various degrees of diarrhea and vomiting (Schaeppi 1974; Pizzolato and Saltz, 2003). As shown in Fig. 2, the mortalities of bolus and infusion groups were dose-related, and the toxicity of CKD-602 by continuous infusion was about 10 times greater than by bolus injection (Table 1). Preclinical studies on CPTs suggested that protracted schedules produce a greater antitumor effect than bolus administration (Thompson *et al.*, 1998; Jung and Zamboni, 2001), indicating the toxic effects of CKD-602 may be dependant upon the duration of intravenous administration.

In terms of changes in body weights in the bolus group, dose-dependant decrease in body weight with increasing dose were observed on 3 days post-treatment (Table 3). In infusion group at 9 mg/kg, rats died within 7 days after treatment. This finding indicated that the body weight decrease was associated with gastrointestinal symptoms, since gastrointestinal symptoms did not occur until 3 days post-treatment.

Taken together, the test item-related and dose-related effects were gastrointestinal symptoms and body weight. In terms of LD_{50} values, CKD-602 was about 10 times more toxic for 24 hr-infusion than for bolus injection. Thus, our findings suggest that 24-hr infusion administration may cause a higher and more protracted test item concentration in target tissues, although the same total drug dose was administered over 24-hr infusion or bolus injection.

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