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Comparison of Single-Dose Toxicity by Intravenous Infusion or Bolus Injection with CKD-602, a Camptothecin Anticancer Agent in Rats (II): Hematological and Serum Biochemical, and Histopathological Changes

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ABSTRACT. The toxicity of CKD-602 was investigated at doses of 3, 9, and 27 mg/kg in rats, when the same total dose of CKD-602 was administered over 24 hr-continuous infusion or bolus injection. At 3 and 9 mg/kg, the 24-hr infusion group showed a more decreased WBC count on day 3, compared with the bolus group. Administration of CKD-602 caused more toxic effects such as the significant decreases of RBC counts, hematocrit, hemoglobin, and platelet count on day 7 post-administarion in the 24-hr infusion group than in the bolus group. Administration of CKD-602 also caused histopathological changes such as extramedullary hemopoiesis of liver and spleen, hyperplasia of femoral bone marrow, and caecal dilation. These toxic effects were more severe in the 24-hr infusion group than in the bolus injection group, indicating that the toxicity of CKD-602 may be dependant upon the duration of administration.

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

INTRODUCTION

Camptothecin (CPT) is a plant antitumor alkaloid isolated from *Camptotheca acuminata* (Wall *et al.*, 1966; Pizzolato and Saltz 2003). Although CPT showed some antitumor activity, its development was halted by poor solubility and unpredictable toxicities such as 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 for 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 (Hertzberg *et al.*, 1989). The CPTs have been reported to cause commonly some adverse effects such as diarrhea and myelosuppression (Pizzolato and Saltz, 2003).

CKD-602 is a new camptothecin derivative anticancer agent developed by 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 formula of (7-[2-(N-isopropylamino)ethyl]-(20S)-camptothecin). CKD-602 is highly water-soluble and has potent anticancer activity against gastric and ovarian cancer. Preclinical pharmacologic evaluation of CKD-602 demonstrated broad anticancer activity against various human tumor cell lines and the results were 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 infusion rather than bolus injection (Tomas *et al.*, 1996;

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Edelman and Karnovsky 1994; Gargosky *et al.*, 1994; Flynn *et al.*, 1993). For cellular and pharmacokinetic improvement of CPTs, preclinical studies suggest that protracted schedules of administration produce greater antitumor effect than bolus administration (Thompson *et al.*, 1998; Jung and Zamboni 2001). It is possible that intravenous infusion of CPTs cause not only greater antitumor efficacy but also adverse effects. However, safety assessment of CPTs has not yet clarified in an animal model with intravenous infusion. For safety evaluation studies of the test article, CKD-602, we utilized an effective rat model by intravenous infusion and compared toxicities by intravenous infusion and by bolus injection, when the same total dose of drug is administered over 24-hr continuous infusion or bolus injection.

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. Then the solution was serially diluted to prepare the test item for administration to the remaining test groups. The dosing solution was prepared immediately before the treatment.

Animal group and preparation for treatment

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 by the modified method (Kim *et al.*, 1996) were assigned to the infusion (I) or the bolus (B) groups with four dose (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). They were given the same total dose over 24 hr-continuous infusion or bolus injection at doses of 3, 9 and 27 mg/kg.

Only healthy animals were assigned to the study. The animals 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).

Necropsy and organ weight

On day 14 after the treatment, complete gross postmortem examinations were performed on all terminated animals euthanized by carbon dioxide overdose. The absolute and relative (organ-to-body weight ratios) weights of following organs were measured in all survivors when they were sacrificed: brain, liver, spleen, heart, thymus, lung, kidneys, and adrenal glands.

Hematological and serum biochemical analysis

The blood samples were drawn from a tail vein at days 1, 3, and 7 after treatment, and the blood samples were collected into CBC bottles containing EDTA-2K (Green Cross Medical Industry, Korea), and were analyzed within 20 minutes in our laboratory. At necropsy, blood samples were drawn from the posterior vena cava by using a syringe with a 24 gauge needle under ether anesthesia. The animals were fasted overnight prior to necropsy and blood collecting. Red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count were determined using a hematological autoanalyzer of T-540 Coulter Counter (Coulter Counter Electronics, USA). To get the sera for serum biochemistry, blood samples were centrifuged at 3,000 rpm for 10 minutes within 1 hour after collection. The sera were stored in the -80°C freezer before they were analyzed. Serum biochemistry parameters including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatine phosphokinase, glucose, total protein, albumin, albumin/globulin ratio, blood urea nitrogen, creatinine, triglyceride, phospholipid, total cholesterol, total bilirubin, calcium, and inorganic phosphorus were evaluated by an autoanalyzer (Shimadzu CL-7200, Shimadzu Co., Japan). Serum electrolytes such as chloride, sodium, and potassium were measured by an ion autoanalyzer (644 Na/K/Cl Analyzer, Ciba-Corning Co., USA).

Histopathological examination

The following tissues were obtained from all animals: abnormal lesions, skin, mammary gland, spleen, pancreas, jejunum, stomach, duodenum, ileum, cecum, colon, mesenteric lymph node, salivary gland, submandibular lymph node, ovaries, uterus, vagina, urinary bladder, epididymides, prostates, seminal vesicles, rectum, kidneys, adrenal glands, liver, sternum, thymus, heart, lung, trachea, esophagus, thyroids (including parathyroids), tongue, aorta, sciatic nerve, skeletal muscle, femur, thoracic spinal cord, Harderian glands, brain, pituitary gland, eyes, and testes. Eyes and testes were preserved in Davidson's fixative and Bouin's fixative,

Table 1. Absolute and relative organ 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(n=8)	I(n=8)	B(n=8)	I(n=7)	B(n=7)	I	B(n=5)	I
Body Wt (g)	310.0 ± 12.3	306.4 ± 19.3	310.0 ± 12.1	274.6 ± 31.3*	297.0 ± 10.7	-	269.0 ± 16.9**	-
Liver	8.51 ± 0.75	8.65 ± 0.76	8.5 ± 0.58	8.37 ± 1.00	8.32 ± 0.59	-	7.99 ± 0.48	-
BW (%)	2.75 ± 0.19	2.83 ± 0.23	2.76 ± 0.16	3.06 ± 0.26	2.81 ± 0.18	-	2.97 ± 0.06	-
Spleen	0.69 ± 0.05	0.76 ± 0.07	0.76 ± 0.09	0.96 ± 0.19	0.77 ± 0.08	-	0.86 ± 0.21	-
BW (%)	0.22 ± 0.02	0.25 ± 0.03	0.25 ± 0.03	0.36 ± 0.09	0.26 ± 0.03	-	0.32 ± 0.09**	-
Heart	1.07 ± 0.06	1.12 ± 0.08	1.09 ± 0.10	1.02 ± 0.12	1.11 ± 0.09	-	0.44 ± 0.10	-
BW (%)	0.35 ± 0.02	0.37 ± 0.02	0.35 ± 0.02	0.37 ± 0.02	0.38 ± 0.04	-	0.38 ± 0.02	-
Thymus	0.34 ± 0.07	0.38 ± 0.08	0.321 ± 0.06	0.26 ± 0.06**	0.24 ± 0.07*	-	0.24 ± 0.08*	-
BW (%)	0.11 ± 0.02	0.12 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.08 ± 0.02	-	0.09 ± 0.02	-
Lung	1.49 ± 0.13	1.50 ± 0.10	1.50 ± 0.13	1.47 ± 0.08	1.48 ± 0.10	-	1.39 ± 0.12	-
BW (%)	0.48 ± 0.03	0.49 ± 0.04	0.48 ± 0.04	0.54 ± 0.05	0.50 ± 0.03	-	0.52 ± 0.03	-
Kidneys	2.10 ± 0.20	2.16 ± 0.18	2.14 ± 0.22	2.02 ± 0.20	2.08 ± 0.11	-	1.91 ± 0.08	-
BW (%)	0.68 ± 0.06	0.71 ± 0.06	0.69 ± 0.05	0.74 ± 0.05	0.70 ± 0.05	-	0.71 ± 0.01	-
Adrenal gl.	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	-	0.06 ± 0.00	-
BW (%)	0.018 ± 0.001	0.019 ± 0.003	0.018 ± 0.003	0.021 ± 0.003	0.018 ± 0.002	-	0.023 ± 0.002**	-

Each value represents as mean ± SD (n=5~8).
*, **Significant difference from each VC group (p<0.05, p<0.01).

respectively. Other tissues were fixed with 10% neutral buffered formalin solution. The tissues were routinely processed, embedded in paraffin, and sectioned at 3~5 μm. The sections were stained with Hematoxylin-Eosin stain for microscopic examination. All organs and tissues taken from all animals were examined microscopically. All gross lesions as defined by the pathologist were also included in the examination.

Statistical analysis

Statistical analyses were performed by comparing the different dose groups with Statistical Analysis Systems (SAS/STAT Version 8.1, Cary, NC, USA). Variance homogeneity was examined using the Bartlett test. If the variance was homogeneous, the data was subjected to one-way analysis of variance (ANOVA) and, if not, they were analyzed by the Kruskal-Wallis non-parametric ANOVA. If either of the tests showed a significant difference among the groups, the data were analyzed by the multiple comparison procedure of the Dunnett's post-hoc test. The level of significance was taken as P<0.05 or 0.01.

RESULT

Organ weights and body weight

CKD-602 by either bolus injection or 24-hr infusion produced the mortalities as follows; In the bolus group, the mortalities were 0/8, 0/8, 1/8, and 3/8, at 0, 3, 9, and 27 mg/kg, whereas those were 0/8, 1/8, 8/8, and 8/8 in the infusion group (data not shown). CKD-602 by either bolus injection or 24-hr infusion produced dose-related effects on body weight. The infusion groups showed a decrease of body weight in more than 3 mg/kg subgroups, whereas body weight of the 27 mg/kg subgroup was statistically significantly suppressed in the bolus groups on day 14 after the bolus injection (data not shown).

In the bolus group, absolute thymus weight was significantly decreased in the 9 mg/kg and 27 mg/kg subgroups, and relative weights of spleen and adrenal gland were significantly increased in the 27 mg/kg subgroup compared to those of the control group (Table 1). In the infusion group, absolute thymus weight was also significantly decreased in the 3 mg/kg subgroup compared to that of the control group (Table 1).

Hematological and serum biochemical changes

As shown in Fig. 1, the dot lines showed fluctuation in hematological parameters of two vehicle control subgroups when the same total dose of CKD-602 was given to intravenous infusion group or intravenous bolus injection group, respectively. Both of the bolus injection and the 24-hr infusion administration decreased WBC counts in a dose-dependant manner within 3 days after treatment. In the bolus group, 3 mg/kg subgroups showed a decrease in WBC counts on day 1 while the 9 and 27 mg/kg subgroup showed a more decreased WBC counts on day 3 (Table 2). In the 24-hr infusion group, 3 and 9 mg/kg subgroups showed a more decreased WBC counts on day 3. In the bolus group, 3 and 9 mg/kg subgroups did not show significant decreases in RBC counts, hematocrit, and hemoglobin on days 1 and 3. However, 3 mg/kg subgroup of in the infusion

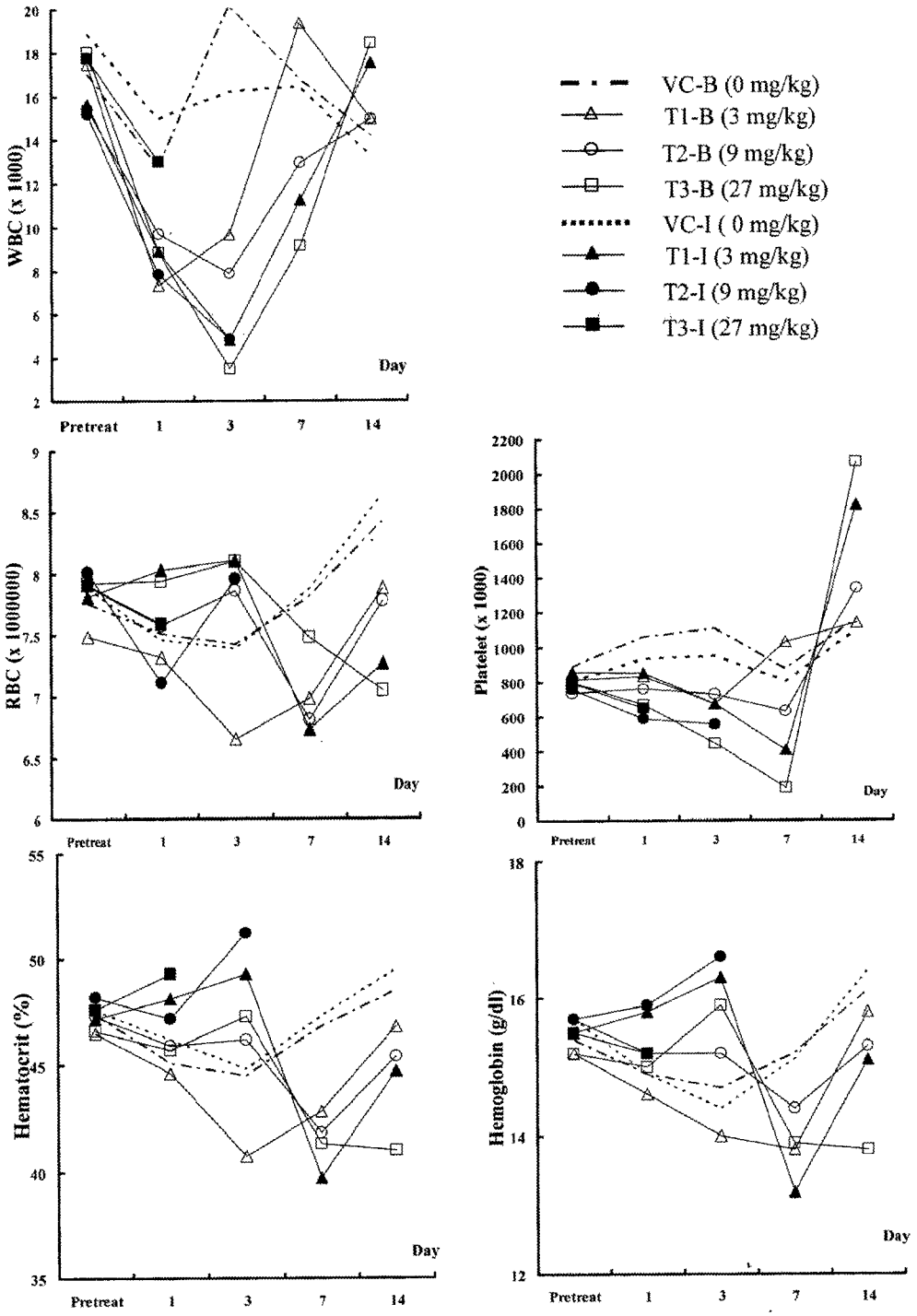

Fig. 1. Hematological changes in rats treated with CKD-602 by bolus injection or 24 hr-infusion. The dot lines showed fluctuation in hematological parameters of two vehicle control subgroups when the same total dose of CKD-602 was given to 24-hr infusion or bolus injection, respectively.

group showed decreases in RBC counts, hematocrit, and hemoglobin on days 3, 7, and 14 after treatment, while 9 mg/kg subgroup showed decreases in RBC counts and hematocrit value on day 3. On day 14, the decreased parameters returned to the same levels as two vehicle control groups (Table 2). Both of the bolus injection and the 24-hr infusion administration greatly increased platelets in a dose-dependant manner within

Table 2. Hematological Changes in rats treated with CKD-602 by bolus injection or 24-hr infusion

	Day	0 mg/kg		3 mg/kg		9 mg/kg		27 mg/kg	
		B	I	B	I	B	I	B	I
WBC	D 1	12.76 ± 3.06	14.96 ± 4.81	7.32 ± 1.65**	8.86 ± 2.07**	9.69 ± 4.69	7.82 ± 3.05*	8.84 ± 1.47*	13.0 ± 3.04
(10^3/μl)	D 3	20.15 ± 2.93	16.19 ± 4.59	9.66 ± 3.09**	4.80 ± 2.74**	7.87 ± 4.51**	4.84 ± 2.29**	3.48 ± 1.66**	-
(17.02	D 7	16.75 ± 4.09	16.41 ± 3.2	19.35 ± 5.79	11.23 ± 2.66**	12.95 ± 4.19	-	9.11 ± 1.64*	-
~18.90)	D 14	14.13 ± 3.85	13.21 ± 4.58	14.91 ± 3.97	17.52 ± 2.67	14.94 ± 3.75	-	18.42 ± 3.50	-
RBC	D 1	7.51 ± 0.23	7.46 ± 0.90	7.32 ± 0.83	8.03 ± 0.58	7.58 ± 0.66	7.11 ± 2.07	7.94 ± 0.87	7.59 ± 0.92
(10^6/μl)	D 3	7.42 ± 0.41	7.39 ± 0.40	6.65 ± 1.25	8.11 ± 0.34**	7.86 ± 0.34	7.96 ± 0.39*	8.10 ± 1.47	-
(7.49	D 7	7.81 ± 0.68	7.87 ± 0.58	6.98 ± 0.62	6.73 ± 0.64**	6.81 ± 0.82	-	7.48 ± 1.04	-
~8.01)	D 14	8.42 ± 0.29	8.63 ± 0.23	7.88 ± 0.49	7.27 ± 0.78**	7.78 ± 0.54	-	7.05 ± 0.67**	-
HGB	D 1	14.9 ± 1.3	14.9 ± 1.1	14.6 ± 1.4	15.8 ± 0.6	15.2 ± 0.5	15.9 ± 0.6	15.0 ± 1.3	15.2 ± 0.4
(g/dl)	D 3	14.7 ± 0.3	14.4 ± 0.8	14.0 ± 1.6	16.3 ± 1.1**	15.2 ± 1.0	16.6 ± 0.7	15.9 ± 1.7	-
(15.2	D 7	15.2 ± 1.1	15.1 ± 0.9	13.8 ± 0.8	13.2 ± 1.1**	14.4 ± 2.2	-	13.9 ± 1.4	-
~15.7)	D 14	16.1 ± 0.6	16.4 ± 0.4	15.8 ± 0.8	15.1 ± 0.9**	15.3 ± 0.7	-	13.8 ± 1.3*	-
HCT	D 1	45.1 ± 1.27	46.2 ± 3.4	44.6 ± 3.8	48.1 ± 2.8	45.9 ± 1.7	47.2 ± 4.7	45.7 ± 4.7	49.3 ± 3.6
(%)	D 3	44.5 ± 2.1	44.8 ± 3.1	40.7 ± 4.4	49.3 ± 2.8*	46.2 ± 3.0	51.2 ± 4.1**	47.3 ± 6.3	-
(46.5	D 7	46.8 ± 3.7	47.3 ± 4.2	42.8 ± 2.3	39.7 ± 3.6**	41.8 ± 3.4*	-	41.3 ± 4.8*	-
~60.5)	D 14	48.5 ± 2.2	49.5 ± 2.0	46.8 ± 2.0	44.7 ± 1.9**	45.4 ± 1.6	-	41.0 ± 3.8**	-
MCV	D 1	60.1 ± 0.8	62.4 ± 6.1	61.1 ± 2.7	60.0 ± 1.64	60.8 ± 4.6	70.3 ± 18.1	57.6 ± 2.9	65.4 ± 5.3
(fl)	D 3	60.0 ± 1.2	60.6 ± 2.1	64.3 ± 13.2	60.8 ± 2.3	58.7 ± 1.8	64.5 ± 6.6	59.0 ± 5.1	-
(58.9	D 7	60.0 ± 1.5	60.1 ± 2.0	61.6 ± 3.0	59.0 ± 1.1	62.3 ± 11.2	-	55.3 ± 3.1	-
~60.5)	D 14	57.6 ± 1.2	57.3 ± 1.5	59.6 ± 2.5	61.8 ± 4.0	58.5 ± 2.6	-	58.2 ± 2.9	-
MCH	D 1	19.8 ± 0.7	20.2 ± 1.86	20.0 ± 1.1	19.7 ± 1.0	20.1 ± 1.5	24.2 ± 8.58	19.0 ± 1.1	20.2 ± 2.6
(pg)	D 3	19.9 ± 1.0	19.4 ± 0.4	22.5 ± 7.5	20.0 ± 0.9	19.4 ± 0.6	20.9 ± 1.6	20.0 ± 2.8	-
(19.2	D 7	19.4 ± 0.7	19.1 ± 0.5	19.9 ± 1.5	19.7 ± 1.0**	21.6 ± 6.3*	-	18.6 ± 1.1**	-
~20.3)	D 14	19.1 ± 0.7	19.0 ± 0.4	20.1 ± 0.7	20.8 ± 1.0**	19.8 ± 0.8	-	19.6 ± 0.5	-
MCHC	D 1	32.9 ± 1.1	32.4 ± 1.5	32.8 ± 0.9	32.9 ± 1.1	33.1 ± 0.8	34.0 ± 3.2	32.9 ± 0.8	30.9 ± 2.9
(g/dl)	D 3	33.2 ± 1.2	32.1 ± 1.3	34.4 ± 3.3	33.0 ± 1.2	33.0 ± 0.6	32.5 ± 1.4	33.9 ± 1.8	-
(32.5	D 7	32.4 ± 1.2	31.9 ± 1.4	32.3 ± 1.7	33.4 ± 1.1	34.3 ± 3.0	-	33.7 ± 0.6	-
~33.1)	D 14	33.2 ± 1.3	33.2 ± 0.6	33.8 ± 0.7	33.7 ± 0.6	33.8 ± 0.6	-	33.7 ± 1.0	-
PLT	D 1	1060 ± 159	930 ± 187	832 ± 150*	852 ± 252	758 ± 211**	586 ± 248	671 ± 174**	649 ± 17.0
(10^3/μl)	D 3	1108 ± 119	949 ± 216	673 ± 180**	673 ± 167*	727 ± 79**	554 ± 77**	445 ± 146**	-
(735	D 7	874 ± 110	803 ± 141	1030 ± 242	406 ± 181**	630 ± 223*	-	188 ± 223**	-
~888)	D 14	1160 ± 94	1097 ± 96	1141 ± 126	1821 ± 505**	1340 ± 245	-	2065 ± 390**	-

Each value represents as mean ± SD (n=4~8).
*, **Significant difference from VC group ($p<0.05$, $p<0.01$). White blood cell count (WBC), Red blood cell count (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Platelet count (PLT).

1 day or 3 days after treatment (Table 2). In the bolus group, 3, 9, and 27 mg/kg subgroups showed increases in platelets 1 day after treatment, while the infusion group showed it in 3 and 9 mg/kg subgroups 3 day after treatment.

Table 3 showed serum biochemical changes. Twenty-seven mg/kg subgroup of the bolus group showed statistically significant decreases in total cholesterol, A/G ratio, albumin, total bilirubin, and chloride, and 9 mg/kg subgroup of the bolus group also showed a decrease in chloride value. The other parameters did not show any change on sacrifice, although we could not know any changes before day 14, since serum biochemical analysis were not carried out on days 1, 3, and 7.

Histopathological changes

The results of histopathological examinations are shown in Table 4. The histopathological changes occurred in the liver, spleen, femur, cecum and testes. In the bolus group, 3 cases (37.5%) of chronic progressive nephropathy and 5 cases (62.5%) of protein cast of kidney, 1 case (12.5%) of extramedullary hemopoiesis of liver, 7 cases (87.5%) of extramedullary hemopoiesis and 1 case (12.5%) of capsula fibrosis of spleen, 5 cases (62.5%) of hyperplasia of femoral bone marrow, and 1 case (12.5%) of seminiferous tubular atrophy were observed in the 3 mg/kg subgroup. Three cases (42.9%) of chronic progressive nephropathy and 5 cases (62.5%) of protein cast of kidney, 3 cases (42.9%) of extramed-

Table 3. Serum biochemical values 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	
	Bolus (B)	Infusion (I)	B	I	B	I	B	I
AST (IU/l)	122.6 ± 16.4	125.9 ± 19.1	116.2 ± 8.2	109.4 ± 21.1	122.8 ± 19.6	-	100.2 ± 19.9	-
ALT (IU/l)	56.4 ± 8.0	55.8 ± 9.1	62.4 ± 10.9	44.7 ± 8.1	57.6 ± 11.1	-	46.8 ± 8.9	-
ALP (IU/l)	459 ± 111	411 ± 116	437 ± 90	479 ± 203	450 ± 81	-	595 ± 243	-
BUN (mg/dl)	22.9 ± 3.4	24.9 ± 3.2	24.6 ± 3.2	22.4 ± 4.5	25.4 ± 6.0	-	24.5 ± 4.0	-
CREA (mg/dl)	0.41 ± 0.04	0.43 ± 0.07	0.43 ± 0.05	0.37 ± 0.07	0.40 ± 0.06	-	0.44 ± 0.10	-
GLU (mg/dl)	118.2 ± 16.5	132.8 ± 36.8	116.1 ± 11.4	110.2 ± 19.7	126.7 ± 19.7	-	146.4 ± 30.0	-
T-CHO (mg/dl)	109.6 ± 14.0	114.2 ± 16.6	125.1 ± 21.1	130.0 ± 16.0	117.5 ± 7.3	-	158.1 ± 27.3**	-
A/G (ratio)	1.76 ± 0.12	1.72 ± 0.15	1.82 ± 0.20	1.52 ± 0.25	1.74 ± 0.08	-	1.51 ± 0.22*	-
TP (g/dl)	6.51 ± 0.28	6.53 ± 0.21	6.29 ± 0.23	6.55 ± 0.72	6.37 ± 0.14	-	6.45 ± 0.31	-
ALB (g/dl)	4.15 ± 0.12	4.13 ± 0.13	4.05 ± 0.16	3.93 ± 0.33	4.04 ± 0.04	-	3.86 ± 0.10**	-
CPK (IU/l)	684 ± 270	626 ± 192	545 ± 172	598 ± 254	608 ± 262	-	343 ± 144	-
T-BIL (mg/dl)	0.13 ± 0.01	0.13 ± 0.02	0.12 ± 0.02	0.11 ± 0.019	0.12 ± 0.02	-	0.10 ± 0.01**	-
IP (mg/dl)	9.32 ± 1.29	9.77 ± 1.56	8.63 ± 0.67	9.34 ± 0.55	9.10 ± 0.62	-	9.02 ± 0.59	-
Ca (mg/dl)	6.80 ± 0.49	7.04 ± 0.69	6.68 ± 0.28	6.48 ± 0.30	6.35 ± 0.23	-	6.58 ± 0.27	-
Cl (mmol/l)	102 ± 1.2	103 ± 1.2	103 ± 1.9	104 ± 0.8*	106 ± 2.2**	-	106 ± 1.0**	-
Na (mmol/l)	143 ± 1.9	143 ± 1.4	143 ± 1.2	142 ± 1.6	143 ± 2.4	-	142 ± 1.8	-
K (mmol/l)	5.21 ± 1.00	5.98 ± 1.61	4.82 ± 0.40	5.75 ± 0.58	5.03 ± 0.46	-	5.36 ± 0.15	-

Each value represents as mean ± SD (n=5-8).
*, **Significant difference from VC group ($p<0.05$, $p<0.01$).
AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphate; BUN, Blood urea nitrogen; CREA, Creatinine; GLU, Glucose; T-CHO, Total cholesterol; A/G, Albumin/globulin; TP, Total protein; ALB, Albumin; CPK, Creatine phosphokinase; TG, Triglyceride; T-BIL, Total bilirubin; IP, Inorganic phosphate.

Table 4. Histopathological changes in 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
Kidney								
Chronic progressive nephropathy	0 (0)	4 (50)	3 (37.5)	3 (42.9)	3 (42.9)	-	4 (80.0)	-
Protein cast	2 (25)	5 (62.5)	5 (62.5)	4 (57.1)	5 (62.5)		3 (60.0)	
Urinary bladder	NPF	NPF	NPF	NPF	NPF	-	NPF	-
Heart	NPF	NPF	NPF	NPF	NPF	-	NPF	-
Liver								
Extramedullary hemopoiesis	0 (0)	0 (0)	1 (12.5)	3 (42.9)	3 (42.9)	-	3 (60)	-
Lung	NPF	NPF	NPF	NPF	NPF	-	NPF	-
Spleen								
Extramedullary hemopoiesis	1 (12.5)	0 (0)	7 (87.5)	6 (85.7)	6 (85.7)	-	5 (100)	-
Fibrosis, capsule	0 (0)	0 (0)	1 (12.5)	0 (0)	1 (14.3)		0 (0)	
Thymus								
Fibrosis	0 (0)	0 (0)	0 (0)	0 (0)	1 (14.3)	-	0 (0)	-
Mesenteric lymph node	NPF	NPF	NPF	NPF	NPF	-	NPF	-
Submandibular lymph node	NPF	NPF	NPF	NPF	NPF	-	NPF	-
Adrenal gland	NPF	NPF	NPF	NPF	NPF	-	NPF	-
Pancreas	NPF	NPF	NPF	NPF	NPF	-	NPF	-
Stomach	NPF	NPF	NPF	NPF	NPF	-	NPF	-
Duodenum	NPF	NPF	NPF	NPF	NPF	-	NPF	-
Jejunum	NPF	NPF	NPF	NPF	NPF	-	NPF	-
Ileum	NPF	NPF	NPF	NPF	NPF		NPF	-
Cecum								
Cystic dilation, mucosa	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-	4 (80)	-
Inflamatory cell infiltration	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		1 (20)	-
Colon	NPF	NPF	NPF	NPF	NPF	-	NPF	-
Femur								
Hyperplasia, bone marrow	0 (0)	0 (0)	5 (62.5)	7 (100)	6 (85.7)	-	5 (100)	-
Testis								
Seminiferous tubular atrophy	0 (0)	0 (0)	1 (12.5)	2 (28.6)	0 (0)	-	4 (80)	-

All tissues taken from all animals (n= 5~8) at necropsy were examined microscopically. NPF represents no pathological finding.

ullary hemopoiesis of liver, 6 cases (85.7%) of extramedullary hemopoiesis and 1 case (14.3%) of capsula fibrosis of spleen, 1 case (14.3%) of fibrosis of thymus, 6 cases (85.7%) of hyperplasia of femoral bone marrow were observed in the 9 mg/kg subgroup. Four cases (80%) of chronic progressive nephropathy and 3 (60%) cases of protein cast of kidney, 3 cases (60%) of extramedullary hemopoiesis of liver, 5 cases (100%) of extramedullary hemopoiesis of spleen, 4 cases (80%) of mucosal cystic dilation and 1 case (20%) of infalmatory cell infiltration of cecum, 5 cases (100%) of hyperplasia of femoral bone marrow, and 4 cases (80%) of seminiferous tubular atrophy were observed in the 27 mg/kg subgroup. In the 24 hr infusion administration group, 3 cases (37.5%) of chronic progressive nephropathy and 4 cases (57.1%) of protein cast of kidney, 3 case (42.9%) of extramedullary hemopoiesis of liver, 6 cases (85.7%) of extramedullary hemopoiesis of spleen, 7 cases (100%) of hyperplasia of femoral bone marrow, and 2 case (28.6%) of seminiferous tubular atrophy were observed in the 3 mg/kg subgroup.

DISCUSSION

In the present study, an effective rat model of continuous intravenous infusion was utilized for the comparison of toxicities between intravenous infusion and bolus injection, when the same total dose of CKD-602 was given to animals. The continuous intravenous infusion has several advantages of intended clinical routes, high bioavailability, and easy assessment of pharmacokinetic parameters under steady-state condition. These methods are available for the rat, dog, mouse and rabbit and have also been applied to primates (Evans and Kerry, 2000). Really, the continuous intravenous infusion had been utilized for many compounds such as IGF-1, heparin, growth hormone, interferon gamma (Tomas *et al.*, 1996; Edelman and Karnovsky 1994; Gargosky *et al.*, 1994; Flynn *et al.*, 1993).

At 27 mg/kg subgroup of the bolus group, a significant increase in relative spleen weight was observed and correlated with high incidence of extramedullary hemopoiesis, and is thus considered to be compound-related change. The decrease of absolute thymus weight and the increase of relative adrenal gland weight observed in the 27 mg/kg subgroup of the bolus group were attributed to the administration of CKD-602, but associated histopathological changes were not observed in the thymus and adrenal glands. Therefore it is considered to be of no toxicological significance. The decreases in thymus weight has been reported to be due to mild stress (Toti *et al.*, 2000), as they were associated with small increase in relative adrenal weight.

The significant decreases of WBC, RBC counts, hematocrit, hemoglobin, and platelet counts on day 7 postadministarion. According to the clinical studies of camptothecins, the principal dose-limiting toxicities are neutropenia, thrmobocytopenia, anemia, alopecia, nausea, vomiting, diarrhea, mucositis, fatigue, and asthenia (Pizzolato and Saltz, 2003; Rothenberg, 1997; Takimoto *et al.*, 1998). Hematogenic effects observed in the present study, including decreased RBC and hemoglobin are in good agreement with the results of above clinical studies. However, the notable increase in platelets observed in the present study is inconsistent with the results of previous studies. Recently, Bozec *et al.* (1998) reported a case of severe thrombocytopenia after the medication of irinotecan. Cass *et al.* (1998) also showed severe thrombocytopenia and/or neutropenia in patients treated with topotecan. This apparent discrepancy may be explained by the differences in test system, dose level, and duration of administration. The significant changes in WBC, RBC counts, hematocrit, hemoglobin, and platelet counts on day 7 postadministration were more severe in 24-hr infusion group than in the bolus group. This finding indicates that the toxic effects of CKD-602 may be dependant upon the longer duration of administration, showing that the toxic potency of CKD-602 was more toxic in continuous administration than that in bolus administration. Preclinical studies for CPTs suggest that protracted schedules produced greater antitumor effect than bolus administration (Thompson *et al.*, 1998; Jung and Zamboni 2001).

The histopathological findings of the present study included extramedullarly hemopoiesis of liver and spleen and hyperplasia of femoral bone marrow. The increased incidence of extramedullary hematopoiesis observed in the spleen and liver indicated that hematopoiesis was stimulated in these rats, as a regenerative response to the anemia. The higher incidence of hyperplasia in femoral bone marrow considered to be a secondary consequence due to the marked bone marrow depression. The CPTs have been reported to cause commonly some adverse effects such as diarrhea and myelosuppression (Pizzolato and Saltz, 2003). Thus, the extramedullary hematopoiesis and hyperplasia of femur bone marrow in the present study seems to be a secondary consequence due to the marked bone marrow depression. The increased extramedullary hematopoiesis has been reported to be related to hematopoietic cell hyperplasia in bone marrow (Fujitani *et al.*, 2004). It has been reported that treatment of rodents with antibiotics causes caecal dilation, probably as a result of changes in caecal microflora (Kasahara *et al.*, 2002; Greaves, 2000).

In the present study, the higher incidence of mucosal cystic dilation in the cecum is considered to be due to CKD-602, probably due to changes in caecal microflora. The other histopathological changes observed in the treatment groups were not considered compound-related effects, because they occurred in a low incidence and the absence of a dose-response relationship.

In conclusion, CKD-602 administration caused an increase in spleen weight, decreases in WBC, RBC, hemoglobin, hematocrit, hemoglobin, and platelet counts, histopathological changes such as extramedullary hemopoiesis of liver and spleen, hyperplasia of femoral bone marrow, and caecal dilation. These toxic effects were more severe in 24-hr infusion than in the bolus administration, indicating that the toxic effects of CKD-602 may be dependant upon the duration of administration.

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REFERENCES

Bleiberg, H. and Rothenberg, M.L. (1996): CPT-11: From DNA topology to clinical activity. *Semi. Oncol.*, **23**, 1-50.

Bozec, L., Bierling, P., Fromont, P., Levi, F., Debat, P., Cvitkovic, E. and Misset, J.L. (1998): Irinotecan-induced immune thrombocytopenia. *Ann. Oncol.*, **9**, 453-455.

Cass, I., Kuo, D.Y., Fields, A.L., Runowicz, C.D. and Goldberg, G.L. (1998): Profound thrombocytopenia in previously treated patients with recurrent ovarian carcinoma receiving topotecan. *Gynecol. Oncol.*, **69**, 175-178.

Dahut, W., Harolod, N., Takimototo, C., Allegra, C., Chen, A., Hamilton, J.M., Arbuck, S., Sorensen, M., Grollman, F., Nakashima, H., Lieberman, R., Liang, M., Corse, W. and Grem, J. (1996): Phase I and pharmacokinetic study of 9-aminocamptothecin given as a 72-hour infusion in adult cancer patients. *J. Clin. Oncol.*, **14**, 1236-1244.

Edelman, E.R. and Karnovsky, M.J. (1994): Contrasting effects of the intermittent and continuous administration of heparin in experimental restenosis. *Circulation*, **89**, 770-776.

Evans, J.G. and Kerry, P.J. (2000): Common pathological findings in continuous infusion studies. In: Guy healing and David Smith (eds.) Handbook of preclinical continuous intravenous infusion. Taylor & Francis, pp 253-264.

Flynn, J.L., Chan, J., Triebold, K.J., Dalton, D.K., Stewart, T.A. and Bloom, B.R. (1993): An essential role for interferon gamma in resistance to mycobacterium tuberculosis infection. *Journal of Experimental Medicine*, **178**, 2249-2254.

Fujitani, T., Tada, Y. and Yoneyama, M. (2004): Chlorpropham-induced splenotoxicity and its recovery in rats. *Food Chem. Toxicol.*, **42**, 1469-1477.

Gargosky, S.E., Tapanainen, P. and Rosenfeld, R.G. (1994): Administration of growth hormone (GH), but not insulin-like growth factor-I (IGF-I), by continuous infection can induced the formation of the 150-kilodalton IGF-binding protein-3 complex in GH-deficient rats. *Endocrinology*, **134**, 2267-2276.

Greaves, P. (2000): Digestive system I, In: Histopathology of preclinical toxicity studies (2nd eds) Elsevier Science, pp. 398-401.

Gottlieb, J.A., Guarino, A.M., Call, J.B., Oliverio, V.T. and Block, J.B. (1970): Preliminary pharmacologic and clinical evaluation of camptothecin sodium (NSC 100880). *Cancer Chemother. Rep.*, **54**, 461-470.

Hertzberg, R.P., Caranfa, M.J., Holden, K.G., Jakas, D.R., Gallagher, G., Mattern, M.R., Mong, S.M., Bartus, J.O., Johnson, R.K. and Kingsbury, W.D. (1989): Modification of the hydroxy lactone ring of camptothecin: Inhibition of mammalian topoisomerase I and biological activity. *J. Med. Chem.*, **32**, 715-720.

Jung, L.L. and Zamboni, W.C. (2001): Cellular, pharmacokinetic, and pharmacodynamic aspects of response to camptothecins: can we improve it ? *Drug. Resistance Updates*, **4**, 273-288.

Kasahara, K., Nishikawa, A., Furukawa, F., Ikezaki, S., Tanakamaru, Z., Lee, I.S., Imazawa, T. and Hirose, (2002): A chronic toxicity study of josamycin in F344 rats. *Food Chem. Toxicol.*, **40**, 1017-1022.

Kim, C.Y., Imai, Y., Ito, K., Hashimoto, J., Nobunaga, T., Satoh, H. and Abe, K. (1996): Analysis of circadian variation of blood pressure and heart rate in dexamethasone-induced hypertensive rats. *Clin. Exper. Hypertension*, **18**, 65-76.

Kolimannsberger, C., Mross, K., Jakob, A., Kanz, L. and Bokemyer, C. (1999): Topotecan - A novel topoisomerase I inhibitor: Pharmacology and clinical experience. *Oncology*, **56**, 1-12.

Lee, J.H., Lee, J.M., Kim, J.K., Ahn, S.K., Lee, S.J., Kim, M.Y., Jew, S.S., Park, J.G. and Hong, C.I. (1998): Antitumor activity of 7-[2-(N-isopropylamino)ethyl]-(20S)- camptothecin, CKD602, as a potent DNA topoisomerase I inhibitor. *Arch. Pharm. Res.*, **21**, 581-590.

Moertel, C.G., Schutt, A.J., Reitmeier, R.J. and Hahn, R.G. (1972): Phase II study of camptothecin (NSC 100880) in the treatment of advanced gastrointestinal cancer. *Cancer Chemother. Rep.*, **56**, 95-101.

Pizzolato, J.F. and Saltz, L.B. (2003): The camptothecins. *Lancet*, **361**, 2235-2242.

Rothenberg, M.L. (1997): Topoisomerase I inhibitors: review and update. *Ann. Oncol.*, **8**, 837-855.

Slichenmyer, W.J. and Rowinsky, E.K. (1993): The current status of camptothecin analogues as antitumor agents. *J. Natl. Cancer Inst.*, **85**, 271-291.

Takimoto, C.H., Wright, J. and Arbuck, S.G. (1998): Clinical applications of the camptothecins. *Biochim. Biophys. Acta*, **1400**, 107-119.

Thompson, J., Stewart, C.F. and Houghton, P.J. (1998): Animal models for studying the action of topoisomerase I targeted drugs. *Biochim. Biophys Acta*, **1400**, 301-319.

Toti, P., De Felice, C., Stumpo, M., Schrfeld, K., Di Leo, L., Vatti, R., Bianciardi, G., Buonocore, G., Seemayer, T.A. and Luzi, P. (2000): Acute thymic involution in fetuses and neonates with chorioamnionitis. *Hum. Pathol.*, **31**, 1121-1128.

Tomas, F.M., Lemmey, A.B., Read, L.C. and Ballard, F.J. (1996): Superior potency of infused IGF-I analogues which bind poorly to IGF-binding proteins is maintained when administered by injection. *J. of Endocrinology*, **150**, 77-84.

Wall, M.E., Wani, M.C. and Cook, C.E. (1966): Plant antitumor agents. 1. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from Camptotheca acuminata. *J. Am. Chem. Soc.*, **88**, 3888-3890.