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Toxicity Study of CKD-602, a Camptothecin Anticancer Agent: 5-Day Repeated Intravenous Administration in Rats

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Abstract – The present study was conducted to investigate the potential subacute toxicity of CKD-602 by a 5-day repeated intravenous administration in Sprague-Dawley rats. CKD-602 was administered intravenously to male rats at dose levels of 0, 0.08, 0.2, and 0.5 mg/kg for 5 days. Studies included general observation, body weight changes, ophthalmoscopic examination, hematology, serum biochemistry, gross findings at necropsy and organ weight measurement. There were no deaths in any treatment group and treatment related clinical sign was depilation in the 0.5 mg/kg groups. The decrease or suppression of body weight was also observed dose-dependently in all treatment groups. Decreased leukocyte in all treatment groups, decreased platelet in the above 0.2 mg/kg groups and increase in the serum levels of total cholesterol in the 0.5 mg/kg group were considered as a treatment related toxic effects. Decreased weight of thymus in all treatment groups and decreased weight of spleen in the above 0.2 mg/kg group were observed. The intravenous administration of CKD-602 caused depilation and decreased weight and had toxic effect on the leukocyte, platelet, spleen and thymus. In the condition of this study, the target organs were spleen and thymus and the toxic effect level was determined to be 0.2 mg/kg, but no-observed-adverse-effect level (NOAEL) was considered to be lower than 0.08 mg/kg.

Keywords □ CKD-602, 5-day repeated intravenous toxicity, Rats

INTRODUCTION

Camptothecin, an alkaloid with a unique heterocyclic ring structure, is extracted from the heartwood of the tree *Camptotheca acuminate* (Wall *et al.*, 1966). Camptothecin inhibited the biosynthesis of nucleic acids in mammalian cells, was found to be potent, rapidly acting inhibitor of topoisomerase I which is an important nuclear enzyme for various DNA synthesis and its functions (Moore *et al.*, 1970; Hertzberg *et al.*, 1989). DNA synthesis inhibiting agents are well known to produce toxic side effects on multiple organ systems (Han *et al.*, 2003). The most common adverse effects associated with Camptothecin are emesis, hemorrhagic diarrhea and myelosuppression (Schaeppi *et al.*, 1974; Kurita *et al.*, 2000; 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 equal or superior to those of other camptothecin analogs (Lee *et al.*, 1998). After a preclinical pharmacologic evaluation, CKD-602 is now under clinical investigation.

In this study we report the results of 5-day repeated intravenous dose toxicity study in Sprague-Dawley rats as a part of the preclinical safety evaluation program for CKD-602. This study was conducted according to the test guidelines from the KFDA and OECD guidelines for the testing of chemicals under modern Good Laboratory Practice Regulations.

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MATERIALS AND METHODS

Test item

CKD-602 (purity≥98.3%) was supplied from Jong 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. The intravenous administration was selected for animal treatment in the present study, because the intravenous route is the intended clinical route for human.

Dosing and dose selection

Healthy males and females were randomly assigned to four experimental groups: Based on the results of the preliminary 5-day repeated intravenous toxicity study, LD_{50} was 2.07 mg/kg and the death was observed in the above 0.67 mg/kg groups. Therefore, 0.5 mg/kg was selected as the high dosage and two other lower dosages were chosen using 2.5 as a common ratio. The vehicle control rats received dissolution solution (100 mg of D-mannitol and 0.12 mg of tartaric acid in 2 ml of distilled water) and the daily volume of administration was calculated according to the most recent body weight. Each group consisted of 5 rats.

Animal treatment

For 5-day subacute toxicity study, twenty-four Sprague-Dawley rats were obtained from Orient Co. (Seoul, Korea) at 6 weeks of age and twenty animals were used after one week of quarantine and acclimatization. The animals were kept in stainless wire cages. Only healthy animals were assigned to the study. An ambient temperature of 25 ± 2°C, relative humidity of 50 ± 2%, and photoperiod of 12 h was maintained throughout the study. The animals were kept in stainless wire cages and were allowed sterilized tap water and commercial rodent chow (Jeil Feed Co, Daejeon, Korea) *ad libitum*. All animal experiments were 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* (NRC, 1996).

Clinical signs, mortality and body weight changes

Through the study, all animals were daily observed for clinical signs of toxicity, moribundity, and mortality. Detailed clinical observations were recorded using Path/Tox System (ver 4.2.2, Xybion Medical Systems Corporation, USA). Body weight of each rat was measured at the initiation of treatment, on the day 1, day 4, and the day of scheduled autopsy.

Hematological and biochemical analysis

Blood samples were drawn from the posterior vena cava using a syringe with a 24 gauge needle under ether anesthesia. 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. Red blood cell count (RBC), hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, white blood cell count (WBC), and differential WBC counts were determined using a hematological autoanalyzer (ADVIA120, Bayer, 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 (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine phosphokinase (CPK), glucose, total protein (TP), albumin, albumin/globulin ratio (A/G ratio), blood urea nitrogen (BUN), 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).

Gross findings and organ weight

Complete gross postmortem examinations were performed on all terminated animals on the next day after final treatment. 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, adrenal glands.

Statistical analysis

Multiple comparison tests for different dose groups were conducted. Variance homogeneity was examined using the Bartlet Test. If the Bartlet Test indicated no significant deviations from variance homogeneity, the ANOVA multiple comparison

test (Dunett Test) was conducted to determine which pairs of group comparison were significantly different. In case that significant deviations from variance homogeneity were observed, a non-parametric comparison test (Kruskal-Walliss(H) Test) was conducted. When a significant difference is observed in the Kruskal-Walliss(H) Test, the Dunn's Rank Test was conducted to determine the specific pairs of group comparison, which are significantly different. For frequency type of data the Chi-square Test was conducted. If a significant difference was found in the Chi-Square Test, the Fisher's Exact Probability Test was conducted to determine the pairs of group comparison, which are significantly different. The level of significance was taken as $P<0.05$ or 0.01. Statistical analyses were performed by comparing the different dose groups with the vehicle control group using Path/Tox System (ver 4.2.2, Xybion Medical Systems Corporation, USA) and Statistical Analysis Systems (SAS/STAT Version 8.1, Cary, NC, USA).

RESULTS

Clinical signs, mortality and body weight changes

There were no treatment-related deaths in any group. There were three incidences of depilation in the 0.5 mg/kg group (data not shown). As shown in Table I, body weight gain in the 0.08 mg/kg group was suppressed and statistically significant body weight decreases were observed in the above 0.2 mg/kg group dose-dependently.

Hematology

On the day 1, decreases in WBC, neutrophil and monocyte in the 0.08 mg/kg group, decreases in WBC, neutrophil and eosinophil in the 0.2 mg/kg group and decreases in the WBC, neutrophil, monocyte, eosinophil and basophil were noted statistically significant (Table II). On the day 5, decreases in RBC, HGB and HCT in the 0.08 mg/kg group, decreases in HCT, MCV, platelet, neutrophil, monocyte and eosinophil in the 0.2 mg/kg group and decreases in WBC, MCV, platelet, neutrophil, lymphocyte, monocyte and eosinophil in the 0.5 mg/kg group were noted statistically significant (Table III).

Table I. Mean body weights in male rats treated with CKD-602 for 5 days

	Dose (mg/kg/day)			
	0	0.08	0.2	0.5
Day 0	327 ± 14.8[a]	327 ± 8.01	321 ± 9.07	325 ± 11.8
Day 1	331 ± 17.4	324 ± 7.36	316 ± 16.7	325 ± 12.4
Day 4	345 ± 16.2	330 ± 8.52	310 ± 14.7*	290 ± 15.4**
Weight gain	18.5 ± 7.36	2.96 ± 3.00	-6.84 ± 10.2	-35.2 ± 6.60

[a]Values are presented as means ± SD (g).
*,**Significant difference at the $p<0.05$ and $p<0.01$ levels, respectively, when compared with the control group.

Table II. Hematological findings in male rats treated with CKD-602 at day 1

Dose (mg/kg/day)	0	0.08	0.2	0.5
Erythrocytes ($\times 10^{12}$/l)	7.56 ± 0.299[a]	7.63 ± 0.317	7.75 ± 0.594	7.52 ± 0.232
Hemoglobin (g/dl)	15.0 ± 0.51	14.9 ± 0.68	14.8 ± 1.05	14.6 ± 0.66
Hematocrit (%)	44.5 ± 1.64	44.1 ± 2.30	44.4 ± 2.92	43.4 ± 1.85
MCV (fl)	58.9 ± 1.88	57.8 ± 0.90	57.4 ± 1.50	57.7 ± 1.63
MCH (pg)	19.8 ± 0.32	19.6 ± 0.22	19.1 ± 0.45	19.4 ± 0.64
MCHC (g/dl)	33.7 ± 0.68	33.9 ± 0.23	33.3 ± 0.45	33.7 ± 0.38
Platelets ($\times 10^9$/l)	1020 ± 78.9	1055 ± 53.6	980 ± 113	982 ± 137
Leukocytes ($\times 10^9$/l)	14.9 ± 4.57	9.80 ± 2.61*	9.37 ± 2.01*	8.91 ± 1.47*
Neutrophils ($\times 10^9$/l)	1.90 ± 0.402	1.36 ± 1.05	1.10 ± 0.442**	0.94 ± 0.271**
Eosinophils ($\times 10^9$/l)	0.15 ± 0.045	0.09 ± 0.057	0.07 ± 0.023**	0.08 ± 0.014*
Basophils ($\times 10^9$/l)	0.10 ± 0.059	0.07 ± 0.050	0.08 ± 0.038	0.02 ± 0.004**
Lymphocytes($\times 10^9$/l)	11.0 ± 4.29	7.88 ± 1.59	7.57 ± 1.98	7.50 ± 1.29
Monocytes ($\times 10^9$/l)	0.65 ± 0.227	0.36 ± 0.022	0.50 ± 0.135	0.34 ± 0.149

[a]Values are presented as means ± SD. MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration
*,**Significant difference at the $p<0.05$ and $p<0.01$ levels, respectively, when compared with the control group.

Table III. Hematological findings in male rats treated with CKD-602 at day 5

Dose (mg/kg/day)	0	0.08	0.2	0.5
Erythrocytes ($\times 10^{12}$/l)	7.51 ± 0.134[a]	6.82 ± 0.183**	7.43 ± 0.475	7.80 ± 0.182
Hemoglobin (g/dl)	14.7 ± 0.23	13.1 ± 0.29*	14.0 ± 0.95	15.1 ± 0.83
Hematocrit (%)	43.2 ± 1.57	37.7 ± 0.94**	39.9 ± 2.43*	42.8 ± 1.70
MCV (fl)	57.5 ± 2.64	55.2 ± 0.86	53.8 ± 1.14**	54.9 ± 1.28*
MCH (pg)	19.6 ± 0.44	19.3 ± 0.16	18.9 ± 0.48	19.4 ± 0.79
MCHC (g/dl)	34.1 ± 0.91	35.0 ± 0.36	35.1 ± 0.44	35.3 ± 0.91
Platelets ($\times 10^9$/l)	1131 ± 109	1054 ± 163	803 ± 87.3**	631 ± 85.3**
Leukocytes ($\times 10^9$/l)	9.14 ± 3.14	5.68 ± 1.59	5.66 ± 1.37	3.37 ± 0.643**
Neutrophils ($\times 10^9$/l)	1.32 ± 0.382	0.18 ± 0.077*	0.09 ± 0.020*	0.05 ± 0.015**
Eosinophils ($\times 10^9$/l)	0.07 ± 0.021	0.02 ± 0.015	0.01 ± 0.000**	0.01 ± 0.004**
Basophils ($\times 10^9$/l)	0.07 ± 0.033	0.04 ± 0.025	0.03 ± 0.019	0.03 ± 0.019
Lymphocytes ($\times 10^9$/l)	7.42 ± 2.86	5.37 ± 1.56	5.49 ± 1.37	3.25 ± 0.608**
Monocytes ($\times 10^9$/l)	0.21 ± 0.092	0.04 ± 0.020	0.02 ± 0.009*	0.01 ± 0.004**

[a]Values are presented as means ± SD. MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration
*,**Significant difference at the $p<0.05$ and $p<0.01$ levels, respectively, when compared with the control group.

Serum biochemistry

The decreases in ALT, ALP, TP, ALB and Na and increases in GLU, TCHO and PL in the 0.5 mg/kg group were noted statistically significant (Table IV).

Gross findings and organ weight

Gross findings at necropsy revealed no test-item related changes in any treatment groups (data not shown). In organ weight measurement, decreases in relative and absolute weight of thymus in the 0.08 mg/kg group, decreases in relative and absolute weight of thymus and decreases in absolute weight of spleen and heart in the 0.2 mg/kg group were observed. The increase in relative weight of brain, decreases in relative and absolute weight of thymus and decreases in absolute weight of

Table IV. Serum biochemical findings in male rats treated with CKD-602 for 5 days

Dose (mg/kg/day)	0	0.08	0.2	0.5
Aspartate aminotransferase (IU/l)	90.9 ± 9.32[a]	94.6 ± 14.1	99.9 ± 19.4	75.1 ± 17.7
Alanine aminotransferase (IU/l)	34.6 ± 5.90	28.3 ± 3.07	27.9 ± 1.53	25.4 ± 4.92**
Alkaline phosphatase (IU/l)	657 ± 169	719 ± 72.0	568 ± 110	453 ± 108*
Blood urea nitrogen (mg/dl)	15.3 ± 2.47	16.2 ± 1.65	15.5 ± 1.90	16.0 ± 1.80
Creatinine (mg/dl)	0.47 ± 0.078	0.39 ± 0.043	0.40 ± 0.075	0.42 ± 0.047
Glucose (mg/dl)	113 ± 12.3	130 ± 25.0	132 ± 5.5	152 ± 21.9**
Total cholesterol (mg/dl)	61.1 ± 10.5	63.6 ± 7.68	72.4 ± 12.5	95.2 ± 13.8**
Total bilirubin (mg/dl)	0.100 ± 0.015	0.099 ± 0.010	0.103 ± 0.015	0.083 ± 0.012
Total protein (g/dl)	6.09 ± 0.368	5.69 ± 0.169	6.04 ± 0.354	5.27 ± 0.290**
Albumin (g/dl)	4.25 ± 0.024	4.05 ± 0.104	4.20 ± 0.152	3.77 ± 0.253**
Creatine phosphokinase (IU/l)	398 ± 146	446 ± 188	651 ± 304	431 ± 158
Triglyceride (mg/dl)	47.6 ± 19.8	26.8 ± 5.51	28.5 ± 4.82	34.4 ± 9.43
Calcium (mg/dl)	9.63 ± 0.338	9.21 ± 0.193	9.42 ± 0.201	9.42 ± 0.619
Inorganic phosphate (mg/dl)	8.45 ± 1.38	7.70 ± 0.411	7.45 ± 0.505	8.01 ± 0.985
Phospholipid (mg/dl)	93.0 ± 14.6	89.0 ± 5.60	94.0 ± 13.2	120 ± 22.3**
Albumin/Globulin (ratio)	2.39 ± 0.533	2.48 ± 0.167	2.30 ± 0.186	2.57 ± 0.408
Sodium (nmol/l)	143 ± 0.84	143 ± 0.55	143 ± 0.45	141 ± 1.48**
Potassium (nmol/l)	5.03 ± 1.16	4.48 ± 0.488	4.35 ± 0.213	5.26 ± 1.51
Chloride (nmol/l)	104 ± 1.52	106 ± 1.10	105 ± 0.71	104 ± 1.34

[a]Values are presented as means ± SD.
*,** Significant difference at the $p<0.05$ and $p<0.01$ levels, respectively, when compared with the control group.

Table V. Absolute and relative organ weights in male rats treated with CKD-602 for 5 days

Dose (mg/kg/day)	0	0.08	0.2	0.5
Brain (g)	1.86 ± 0.057[a]	1.89 ± 0.070	1.83 ± 0.080	1.85 ± 0.065
Per body weight (%)	0.587 ± 0.029	0.632 ± 0.041	0.650 ± 0.006	0.672 ± 0.033**
Liver (g)	8.95 ± 0.950	8.41 ± 0.333	8.25 ± 1.12	8.71 ± 0.742
Per body weight (%)	2.82 ± 0.224	2.81 ± 0.070	2.92 ± 0.310	3.16 ± 0.250
Spleen (g)	0.628 ± 0.089	0.537 ± 0.035	0.476 ± 0.072*	0.430 ± 0.097**
Per body weight (%)	0.198 ± 0.026	0.179 ± 0.012	0.168 ± 0.020	0.157 ± 0.041
Heart (g)	1.13 ± 0.111	1.06 ± 0.095	0.950 ± 0.037**	0.956 ± 0.059*
Per body weight (%)	0.356 ± 0.029	0.355 ± 0.028	0.337 ± 0.003	0.348 ± 0.021
Thymus (g)	0.445 ± 0.070	0.155 ± 0.034**	0.091 ± 0.032**	0.101 ± 0.026**
Per body weight (%)	0.140 ± 0.018	0.052 ± 0.013**	0.032 ± 0.011**	0.037 ± 0.009**
Lung (g)	1.27 ± 0.040	1.26 ± 0.059	1.20 ± 0.040	1.14 ± 0.069**
Per body weight (%)	0.402 ± 0.011	0.420 ± 0.030	0.424 ± 0.006	0.415 ± 0.029
Kidneys (g)	2.35 ± 0.332	2.14 ± 0.140	2.00 ± 0.199	2.03 ± 0.172
Per body weight (%)	0.741 ± 0.089	0.713 ± 0.038	0.708 ± 0.049	0.737 ± 0.065
Adrenal glands (g)	0.053 ± 0.0082	0.052 ± 0.0052	0.051 ± 0.0034	0.051 ± 0.0052
Per body weight (%)	0.017 ± 0.0027	0.017 ± 0.0015	0.018 ± 0.0006	0.019 ± 0.0024

[a]Values are presented as means ± SD.
*,**Significant difference at the $p<0.05$ and $p<0.01$ levels, respectively, when compared with the control group.

spleen, heart and lung in the 0.5 mg/kg group were observed (Table V).

DISCUSSION

CKD-602, a camptothecin alkaloid analog with a unique heterocyclic ring structure from the heartwood of the tree *Camptotheca acuminate*, is an anticancer agent with high water solubility and has potent anticancer activity against gastric and ovarian cancer. In order to investigate the repeated intravenous toxicity of CKD-602, the test item was administered intravenously to groups of 5 SD rats at doses of 0, 0.08, 0.2 and 0.5 mg/kg for 5 days. Studies included clinical observation, body weight changes, hematology, serum biochemistry, gross findings at necropsy and organ weight measurements. The treatment related death was not found in any treatment groups. The depilation in the 0.5 mg/kg was considered as a treatment related clinical sign because of its high frequency. The suppressed body weight gain in the 0.08 mg/kg group and decreased body weight in the above 0.2 mg/kg groups observed dose-dependently from the first day of treatment were also considered as treatment-related. The dose-dependent decreases in WBC (neutrophil, lymphocyte, monocyte and eosinophil), MCV and platelet were considered as a treatment-related toxic effect considering that CKD-602 is an anticancer agent affecting on the highly proliferating organs such as hematopoietic systems. These hematopoietic toxicity have been already reported on the other camptothecin anticancer agents (Pizzolato and Saltz, 2003; Prijovich *et al.*, 2002). The decreases in ALT and ALP were not toxicologically meaningful, but the increase in TCHO was considered as a treatment-related toxic effect because it was dose-dependent and statistically significant. Some sporadic changes in clinical biochemistry indices were considered as accidental because this were not dose-dependent and within the normal physiological ranges of SD rats (Wolford *et al.*, 1986; Kang *et al.*, 1995). The decreased weights of thymus and spleen were considered as treatment-related because it was dose-dependent and this result coincided with the previous report of 28-day repeated oral dose toxicity study in rats (unpublished data). Camptothecin derivatives are cancer chemotherapeutic agents suppressing the topoisomerase I enzyme and have various adverse effects on the organs such as bone marrow, spleen, mucosal membrane and reproductive organs (Prijovich *et al.*, 2002). The decreased organ weights of spleen and thymus observed in the present study were considered as the specific character of cancer chemotherapeutic agents. The increase in relative weight of brain and decrease in absolute weights of heart and lung in the 0.5 mg/kg group were thought to be a result of decreased body weight rather than toxic effect of CKD-602.

Based on these results, it was concluded that the 5-day repeated intravenous dose of CKD-602 caused depilation and decreased body weight and had toxic effect on the leukocyte, platelet, spleen and thymus. In this study, the target organs were

spleen and thymus and the toxic effect level was determined to be 0.2 mg/kg, but no-observed-adverse-effect level (NOAEL) was considered to be lower than 0.08 mg/kg.

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