J. Toxicol. Pub. Health
Vol. 20, No. 1, pp. 83-88 (2004)

Official Journal of
Korean Society of Toxicology
Available online at http://www.toxmut.or.kr

5-Day Repeated Intravenous Dose Toxicity Study of a New Camptothecin Anticancer Agent CKD-602 in Rats

Jong-Choon Kim[1], Dong-Ho Shin[1], Sung-Ho Kim[1], Chun-Sik Bae[1], Joon-Kyum Kim[2], Shin-Woo Cha[3], Junghee Han[3], Hyun-Sook Lee[3] and Moon-Koo Chung[3]

[1]College of Veterinary Medicine, Chonnam National University, Gwangju 500-757
[2]Medicinal Research Center, Chong Kun Dang Co., Seoul 152-070
[3]Korea Institute of Toxicology, P.O. Box 107, Yuseong, Daejeon 305-600, Korea

Received January 9, 2004; Accepted February 2, 2004

ABSTRACT. The present study was carried out to investigate the potential adverse effects of CKD-602 by a 5-day repeated intravenous dose in Sprague-Dawley rats. The test article, CKD-602, was administered intravenously to male and female rats at dose levels of 0.07, 0.22, 0.67, 2.0 and 6.0 mg/kg/day for 5 days consecutively. Mortalities, clinical findings, and body weight changes were monitored for the 14-day period after cessation of the administration. At the end of 14-day observation period, all animals were sacrificed and complete gross postmortem examinations were performed. There were 2 and 5 treatment related deaths in the 0.67 and 2.0 mg/kg/day dose groups of both genders, respectively. Treatment related clinical signs, including hair loss, skin paleness, decreased locomotor activity, emaciation, and changes in stool were observed in a dose-dependent manner from the third day after initiation of the injection. Decrease or suppression of body weight was also observed dose-dependently in males and females of the treated groups. Gross postmortem examinations revealed a dose-dependent increase in the incidence and severity of atrophy or hypertrophy and white membrane formation in the spleen, atrophy of the thymus, diffuse white spots and paleness of the liver, paleness of the lung, kidney and adrenal gland, and dark red discoloration and dark red contents in the alimentary tract. Based on these results, it was concluded that the 5-repeated intravenous injection of CKD-602 to male and female rats resulted in increased incidence of abnormal clinical signs and death, decreased or suppressed body weight, and increased incidence of abnormal gross findings. In the present experimental conditions, the LD_{50} value was 2.07 (95% confidence limit not specified) mg/kg/day in both genders and the LD_{10} value was 1.72 (95% confidence limit not specified) mg/kg/day in both genders.

Keywords: Anticancer agent, CKD-602, Camptothecin, Repeated dose 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 (Hertzberg *et al.*, 1989). 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

Correspondence to: Jong-Choon Kim, *Department of Toxicology, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Republic of Korea*
E-mail: toxkim@chonnam.ac.kr

damaging agents are well known to produce toxic side effects on multiple organ systems (Kim *et al.*, 2003b, 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.*, 2002). 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 (Lee *et al.*, 1998, 2000; Kim *et al.*, 2003a). 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 5-day repeated intravenous dose toxicity study was performed in Sprague-Dawley rats. This study will provide useful information for assessing toxicological relevance of a camptothecin CKD-602 by repeated administration in clinical trials.

MATERIALS AND METHODS

Animal Husbandry and Maintenance

Thirty-six Sprague-Dawley rats of each sex were obtained from the Korea Institute of Toxicology at 4 weeks of age and used after 2 weeks 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 (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.

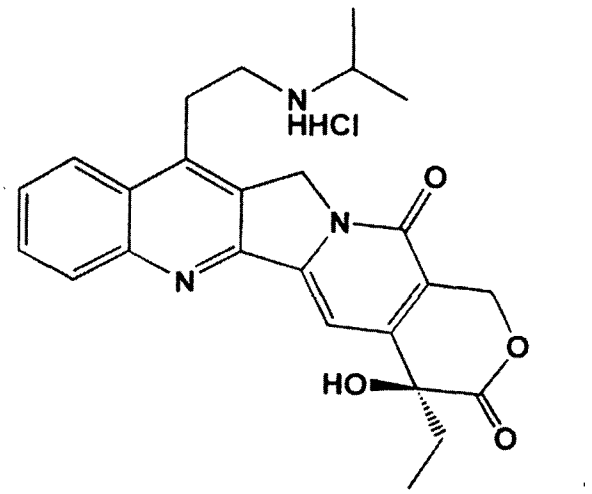

Fig. 1. Chemical structure of CKD-602.

Experimental 1

Healthy males and females were randomly assigned to five experimental groups receiving 0.07, 0.22, 0.67, 2.0, and 6.0 mg/kg/day. Each group consisted of 5 rats of each sex.

Selection of Doses

Our previous toxicity study showed that the acute intravenous LD_{50} ranged from 53 to 60 mg/kg and LD_{10} was >39 mg/kg in rats (unpublished data). Based on the results of the acute toxicity study and a preliminary repeated dose toxicity study, 6.0 mg/kg/day was selected as the highest dose and doses of 2.0, 0.67, 0.22, and 0.07 mg/kg/day were selected as lower doses using a scaling factor of ×3.

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. After 5 daily intravenous doses, the recovery period for 14 days was permitted.

Mortality and Clinical Observation

Clinical signs and mortality were checked once a day

up to day 14 after last dosing. 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, on day 3 of treatment, and on days 1, 3, 7 and 14 after treatment thereafter.

Necropsy

On day 14 after last dosing, 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 analysed 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 5-day repeated intravenous injection are presented in Table 1. There were 2 and 5 deaths in the 0.67 and 2.0 mg/kg dose groups of both genders, respectively. The onset time of death was as follows: in the 6.0 mg/kg dose group, one case on the third day of the last injection and four cases on the fourth day; and in the 2.0 mg/kg dose group, one case on the second day, three cases on the third day, one case on the fourth day and one case each on the sixth and eighth day. On the basis of the results, LD_{50} was 2.07 (95% confidence limit not specified) mg/kg in both genders. LD_{10} was 1.72 (95% confidence limit not specified) mg/kg in both genders.

Table 1. Mortality in male and female rats treated with CKD-602 for 5 days

Dose (mg/kg)	0.07	0.22	0.67	2.0	6.0
Days 0~5	0/0[a)]	0/0	0/0	0/0	0/0
Day 6	0/0	0/0	0/0	0/0	0/1
Day 7	0/0	0/0	0/0	0/0	1/3
Day 8	0/0	0/0	0/0	0/0	4/1
Day 9	0/0	0/0	0/0	2/0	0/0
Day 10	0/0	0/0	0/0	0/1	0/0
Day 11	0/0	0/0	0/0	0/0	0/0
Day 12	0/0	0/0	0/0	0/1	0/0
Days 13~18	0/0	0/0	0/0	0/0	0/0
Total	0/0	0/0	0/0	2/2	5/5

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

Table 2. Clinical findings in male and female rats treated with CKD-602 for 5 days

Dose (mg/kg)	0.07	0.22	0.67	2.0	6.0
Emaciation	0/0[a)]	0/0	0/0	5/3	5/5
Paleness of skin color	0/0	0/0	0/0	2/2	2/0
Dyspnea	0/0	0/0	0/0	1/0	0/0
Decreased locomotor activity	0/0	0/0	0/0	3/1	4/4
Dark material around eyes	0/0	0/0	0/0	3/1	0/0
Dark material around nose	0/0	0/0	0/0	1/0	0/0
Hair loss	0/0	5/4	5/5	5/5	5/5
Soft stool	0/0	0/0	0/0	5/4	0/1
Watery stool	0/0	0/0	0/0	0/0	4/4
Soiled fur	0/0	0/0	0/0	1/0	0/0
Death	0/0	0/0	0/0	2/2	5/5

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

Clinical Findings

As shown by the data in Table 2, treatment-related clinical signs, including hair loss, skin paleness, skin ulcers, emaciation and changes in stool were observed at dose levels of 0.22 mg/kg or greater. Hair loss was observed from the third day of injection in the 6.0 mg/kg dose group of both genders. Stool changes were observed on the 1st day after cessation of the injection. As the symptoms aggravated, emaciation and paleness occurred and finally lead to death. In the 2.0 mg/kg dose group, hair loss was observed on the 3rd day of injection. Soft stool was observed on the 1st day after the injection stopped, and on the 3rd day emaciation and a decrease in locomotor activity were seen. Those who showed aggravating symptoms finally died, and the others showed skin paleness, dark red material around the eyes, dyspnea and soiled fur recovering from the 10th day. In the 0.67 and 2.0 mg/kg groups, hair loss was observed, and no abnormal symptoms were found in the 0.07 mg/kg dose group.

Body Weight Changes

As shown in Table 3, body weights of both sexes at 0.07 mg/kg showed no treatment-related weight changes and were within the limits of normal biological variation (Song *et al.*, 1990). On the contrary, body weights of both sexes at over 0.22 mg/kg were decreased or suppressed in a dose-dependent manner. The body weight reduction observed in females was completely recovered at term, but the change observed in males was not recovered until the end of recovery period.

Gross Findings

The results of gross postmortem examinations are shown in Table 4. Major findings were atrophy or hyper-

Table 3. Body weights in male and female rats treated with CKD-602 for 5 days

Dose (mg/kg)	0.06	0.22	0.67	2.0	6.0
Male					
Day 0	199.6 ± 17.95	193.2 ± 11.63	197.1 ± 7.44	192.8 ± 14.00	197.8 ± 10.36
Day 2	213.3 ± 16.96	202.1 ± 12.47	202.9 ± 7.30	192.4 ± 11.43	189.9 ± 8.69
Day 5	232.6 ± 15.89	216.2 ± 10.52	192.7 ± 12.11	158.3 ± 10.95	149.3 ± 4.48
Day 11	281.7 ± 17.29	256.6 ± 12.22	233.8 ± 21.24	158.3 ± 14.78	
Day 18	331.4 ± 24.87	310.7 ± 14.55	296.2 ± 18.93	187.9 ± 55.47	
Female					
Day 0	154.2 ± 13.53	155.6 ± 9.95	148.0 ± 11.63	155.9 ± 8.25	152.1 ± 6.34
Day 2	158.0 ± 10.12	153.2 ± 9.44	151.4 ± 8.56	152.3 ± 6.33	143.5 ± 2.77
Day 5	165.1 ± 11.75	157.5 ± 10.58	147.5 ± 11.88	137.3 ± 2.87	117.7 ± 2.65
Day 11	184.6 ± 17.93	182.8 ± 12.25	178.0 ± 13.00	148.8 ± 18.59	
Day 18	198.3 ± 15.42	197.6 ± 11.41	202.9 ± 15.26	193.7 ± 8.43	

Values are means± SD (g).

Table 4. Gross findings in male and female rats treated with CKD-602 for 5 days

Dose (mg/kg)		0.06	0.22	0.67	2.0	6.0
Thymus:	atrophy	0/0[a)]	0/0	0/0	4/2	0/5
Lung:	paleness	0/0	0/0	0/0	1/2	0/0
Liver:	paleness	0/0	0/0	0/0	2/2	0/0
	diffuse white spots	0/0	0/0	0/0	1/1	0/0
Spleen:	enlargement	0/0	0/0	0/0	1/0	0/0
	white colored membrane	2/0	2/0	4/2	2/2	0/0
	atrophy	0/0	0/0	0/0	1/0	2/5
	adhesion with fat tissue	0/0	0/0	0/1	0/0	0/0
	vesicles on the surface	0/0	0/0	0/0	0/1	0/0
Kidney:	paleness	0/0	0/0	0/0	0/2	0/0
	diffuse white spots	0/0	0/0	0/0	1/0	0/0
Adrenal gland:	paleness	0/0	0/0	0/0	0/2	0/0
Stomach:	dark red discoloration	0/0	0/0	0/0	0/0	0/2
	diffuse white spots	0/0	0/0	0/0	1/0	0/0
	dark red discoloration	0/0	0/0	0/0	1/0	1/0
	dark red contents	0/0	0/0	0/0	1/0	1/4
Small intestine:	dark red discoloration	0/0	0/0	0/0	1/0	0/0
	dark red contents	0/0	0/0	0/0	1/0	0/0
	hardness	0/0	0/0	0/0	1/0	0/0
	edema	0/0	0/0	0/0	1/0	0/3
Large intestine:	dark red contents	0/0	0/0	0/0	1/0	0/0
Cecum:	enlargement	0/0	0/0	0/0	0/0	1/0
	dark red discoloration	0/0	0/0	0/0	1/0	2/2
	edema	0/0	0/0	0/0	0/0	0/3
Testis:	atrophy	0/-	0/-	0/-	3/-	0/-
	dark red discoloration	0/-	0/-	0/-	1/-	0/-

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

trophy and white membrane formation in the spleen, atrophy of the thymus, diffuse white spots and paleness of the liver, paleness of the lung, kidney and adrenal gland, and dark red discoloration and dark red contents in the alimentary tract.

In males, white membrane formation of the spleen was observed in 2, 4, 2 and 2 cases of those necropsied in the 2.0, 0.67, 0.22 and 0.07 mg/kg groups, respectively. Atrophy of the spleen was found in the 6.0 and 2.0 mg/kg groups, 2 and 1 cases each. Atrophy of the thymus occurred in 4 cases in the 2.0 mg/kg group. Paleness of the liver occurred in 1 case in the 2.0 mg/kg group. Dark red content in the stomach was found in 1 case from the 6.0 mg/kg group and 1 case from the 2.0 mg/kg group. Dark red content in the small bowel was in 1 case in the 2.0 mg/kg group; dark red discoloration of the stomach was in 1 case from the 6.0 mg/kg dose group and 1 case from the 2.0 mg/kg dose group; dark red discoloration of the cecum was found in 2 and 1 cases from the 6.0 and 2.0 mg/kg groups, respectively; and dark red discoloration of the colon was found in 1 case of the 2.0 mg/kg group.

In females, white membrane formation of the spleen was in 2 cases from the 2.0 and 0.67 mg/kg groups. Atrophy of the spleen was in 5 cases from the 6.0 mg/kg group. Paleness of the liver was in 2 cases of the 2.0 mg/kg group, and diffuse white spots on the liver were in 1 case of the 2.0 mg/kg group. Paleness of the lung, adrenal gland and kidney were found in 2 cases from the 2.0 mg/kg dose group, and atrophy of the thymus occurred in 5 and 2 cases in the 6.0 and 2.0 mg/kg group, respectively. Dark red content in the stomach was found in 4 cases of the 6.0 mg/kg group, dark red discoloration of the stomach occurred in 2 cases of the 6.0 mg/kg group, dark red discoloration of the cecum occurred in 1 case of the 6.0 mg/kg group, and edema of the small and large bowels happened in 3 cases of the 6.0 mg/kg group.

DISCUSSION

The present study was conducted to determine the potential adverse effects of CKD-602 by a 5-day repeated intravenous dose in Sprague-Dawley rats. The test article was administered intravenously to male and female rats at dose levels of 0, 0.07, 0.22, 0.67, 2.0

and 6.0 mg/kg/day for 5 days consecutively. Clinical signs, mortality, body weight changes and gross findings were observed for fourteen days after cessation of the injection.

Treatment-related clinical signs, as evidenced by dose-dependent increases in the incidence and severity of hair loss, watery diarrhea, decreased locomotor activity, skin paleness and emaciation, were observed at dose levels of 0.22 mg/kg/day or greater. These clinical signs were observed from the 3rd day of injection. Treatment-related death was observed from the 2nd day after the cessation of injection and was observed in the both sexes of the groups given over 2.0 mg/kg/day of the test article among those showing aggravation of the above clinical signs.

The dose-dependent suppression or reduction of body weights with increasing dose indicates that this finding is caused by the administration of CKD-602. This effect observed in females was completely reversible during the recovery period but the body weight reduction observed in males was not recovered until the end of recovery period.

An increase in the incidence 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. Hypertrophy and white membrane formation of the spleen are considered to be compensatory changes during recovery. Paleness of the solid organs is thought to be a secondary effect of damage to the hematogenic system and alimentary tract hemorrhage.

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 to have toxic effects on highly proliferating organs and tissues.

Based on these results, it was concluded that the 5-repeated intravenous injection of CKD-602 to male and female rats resulted in increased incidence of abnormal clinical signs and death, decreased or suppressed body weight, and increased incidence of abnormal gross findings. In the present experimental conditions, the LD_{50} value was 2.07 (95% confidence limit not specified) mg/kg/day in both genders and the LD_{10} value was 1.72 (95% confidence limit not specified) mg/kg/day in both genders. The doses correspond to about 104 and 86 times of the anticipated human clinical dose of CKD-602 (i.e. 0.02 mg/kg/day), respectively.

REFERENCES

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

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.

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.

Hashimoto, H., Chatterjee, S. and Berger, N.A. (1995): Mutagenic activity of topoisomerase I inhibitors, *Clin. Cancer Res.*, **1**, 369-376.

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.

Kim, J.H., Lee, S.K., Lim, J.L., Shin, H.J. and Hong, C.I. (2002): Preformulation studies of a novel camptothecin anticancer agent, CKD-602: physicochemical characterization and hydrolytic equilibrium kinetics, *Int. J. Pharm.*, **239**, 207-211.

Kim, E.J., Lee, R.K., Suh, J.E., Han, S.S. and Kim, J.K. (2003a): Safety pharmacology of CKD-602, a novel anticancer agent, *Arzneimittel Forschung*, **53**, 272-279.

Kim, J.C., Kim, K.H. and Chung, M.K. (1999): Testicular cytotoxicity of DA-125, a new anthracycline anticancer agent, in rats, *Reprod. Toxicol.*, **13**, 391-397.

Kim, J.C., Kim, S.H., Shin, D.H., Ahn, T.H., Kim, H.C., Kim, Y.B., Jiang, C.Z., Han, J. and Chung, M.K. (2004): Effects of prenatal exposure to the environmental pollutant 2-bromopropane on embryo-fetal development in rats, *Toxicology*, **196**, 77-86.

Kim, J.C., Shin, D.H., Ahn, T.H., Kang, S.S., Song, S.W., Han, J., Kim, C.Y., Ha, C.S. and Chung, M.K. (2003b): 26-Week repeated oral dose toxicity study of the new quinolone antibacterial DW-116 in Sprague-Dawley rats, *Food Chem. Toxicol.*, **41**, 637-645.

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)- camp-

tothecin, CKD602, as a potent DNA topoisomerase I inhibitor, *Arch. Pharm. Res.*, **21**, 581-590.

Lee, J.H., Lee, J.M., Lim, K.H., Kim, J.K., Ahn, S.K., Bang, Y.J. and Hong, C.I. (2000): Preclinical and phase I clinical studies with CKD-602, a novel camptothecin derivative, *Ann. N. Y. Acad. Sci.*, **922**, 324-325.

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.

Pratesi, G., Tortoreto, M., Corti, C., Giardini, R. and Zunino, F. (1995): Successful local regional therapy with topotecan of intraperitoneally growing human ovarian carcinoma xenografts, *Br. J. Cancer*, **71**, 525-528.

SAS Institute, Inc, (1997): *SAS/STAT Software: Changes and Enhancements Through Release 6.12.* SAS Institute, Cary, NC.

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

Song, C.W., Hwang, H.S. and Han, S.S. (1990): Studies on the basic data of Ktc: SD rats with age, *Korean J. Lab. Ani. Sci.*, **6**, 33-43.

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