STEALTH® Liposomal CKD-602, a Topoisomerase I Inhibitor, Improves the Therapeutic Index in Human Tumor Xenograft Models

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Abstract. *Background: CKD-602, a topoisomerase I inhibitor, has antitumor activity in a broad spectrum of tumor types. STEALTH® liposomal CKD-602 (S-CKD602) prolongs circulation of CKD-602 in plasma, increases drug exposure in tumors and improves efficacy compared with free drug. Materials and Methods: Different dosing regimens of S-CKD602, free CKD-602 and topotecan were compared for antitumor activity in female athymic nude mice bearing human A375 melanoma, ES-2 ovarian, H82 SCLC or HT-29 colon tumor xenografts. Results: S-CKD602 was more efficacious than free drug in all tumor types studied. The therapeutic index (TI) of S-CKD602 was estimated to be ~6-fold greater than that of free CKD-602 in ES-2 and ~3-fold greater in H82 tumors. TI of S-CKD602 was ~2-fold greater than that of free CKD-602 and ~5-fold greater than that of topotecan in A375, and ≥3-fold greater in HT-29 tumors. In A375 tumors, once-weekly dosing of S-CKD602 was superior to once every 2 weeks or twice weekly schedules. Conclusion: The therapeutic index of S-CKD602 was greater than that of free CKD-602 and topotecan in several human tumor types.*

CKD-602 ((20S)-7-(2-isopropylamino)-ethylcamptothecin hydrochloride), a topoisomerase I inhibitor, is a semi-synthetic, water-soluble analog of camptothecin developed by Chong Kun Dang Pharmaceutical Corporation in Seoul, South Korea (1). CKD-602 has demonstrated antitumor activity in a broad spectrum of human tumor types including ovarian, lung, colon, and mammary carcinomas, and was more efficacious than topotecan (2-3). Because this class of drug is most effective in the S-phase of the cell cycle, frequent dosing is necessary to maintain effective plasma concentrations.

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The STEALTH® liposomal delivery system (ALZA Corporation, Mountain View, CA, USA) is used to encapsulate CKD-602 in an acidic condition and preserve the lactone ring required for its antitumor activity. When administered parenterally, STEALTH® liposomes can evade detection by the mononuclear phagocytic system (MPS) and provide longer circulation time because of surface-bound methoxypolyethylene glycol (MPEG), which is covalently linked to 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE). The long half-life and small size of the liposomes (approximately 100 nm in diameter) allows selective accumulation in tumor interstitial tissue *via* the compromised vasculature formed during the angiogenesis process (4-6). Using this drug delivery technology, S-CKD602 can be developed as an oncologic product if improvement in efficacy and/or safety can be demonstrated.

In this study, the antitumor activity of S-CKD602 was evaluated in four human tumor xenograft models and compared to that of non-liposomal or free CKD-602 and topotecan. In addition, the therapeutic index (TI) of S-CKD602 was evaluated for various treatment schedules.

Materials and Methods

Drug formulations. The free CKD-602 solution (2 mg/mL) contained tartaric acid and mannitol in 5% dextrose. Lyophilized topotecan (4 mg/vial) was obtained from a commercial source (GlaxoSmithKline, Research Triangle Park, NC, USA) and reconstituted in sterile water prior to use. S-CKD602 was a sterile, clear to slightly opalescent suspension (0.1 mg/mL CKD-602). The STEALTH® liposomes were composed of MPEG-DSPE and 1,2-distearoyl-sn-glycero-phosphocholine (DSPC) in a 5:95 molar ratio. The average liposome diameter was 100 nm and drug encapsulation was 96%.

Animals. Female NCR.nu/nu homozygous mice (Harlan Laboratories, Indianapolis, IN, USA), approximately 5-6 weeks old, were maintained in isolator cages on a 12-hour light-and-dark cycle. Food and water were available *ad libitum*. All studies were performed in compliance with animal welfare regulations and the Guide for the Care and Use of Laboratory Animals in an AAALAC internationally accredited facility.

Tumor cells and xenografts preparation. A375 human melanoma, ES-2 human ovarian, H82 small cell lung cancer (SCLC) and HT-29 human colon carcinoma cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained in growth medium, as appropriate, at 37˚C in a humidified 5% CO_2 incubator. Log-phase tumor cells were harvested from culture flasks and injected ($5x10^6$ cells in 100 μL) subcutaneously in flank. When tumors reached approximately 100 mm^3 in size, animals were randomly assigned to treatment groups.

Study design. There were 14 separated studies in this report: 5 in A375, 3 in ES-2, 2 in H82 SCLC and 4 in HT-29. Five to twelve animals were used per treatment groups and control group in each study. In some cases a particular dosing regimen, usually served as a positive comparator, was included in more than one study. S-CKD602 (0.1 to 3.5 mg/kg) was administered intravenously (*i.v.*) and free CKD-602 was administered either *i.v.* (10 to 35 mg/kg) or intraperitoneally (*i.p.*, 3 to 20 mg/kg). Topotecan was given *i.p.* (3 to 20 mg/kg). In all 4 xenograft models, both S-CKD602 and free CKD-602 were given once weekly (*qw*) *i.v.* In both A375 and HT-29 models, free CKD-602 and topotecan were also given once every 4 days (*q4d*) *i.p.* In A375, S-CKD602 was also given twice weekly (*biw*) and once every 2 weeks (*q2w*) to evaluate the influence of dosing schedules on antitumor activity. Details of the dosing regimens were listed in Tables II to V.

Antitumor efficacy. Tumors were measured 2 to 3 times a week for 30 days after the last dosing or until they quadrupled in size. Tumor volume was calculated according to the formula:
$V = 1/2 \times D_1 \times D_2 \times D_3$,
where D_{1-3} are three perpendicular diameters measured in millimeters (mm). Tumor volume quadrupling time (TVQT), defined as the time required for a tumor to reach four times (4x) its initial treatment volume, was used as study endpoint. Within each experiment, a statistical analysis (Student's *t*-test) on TVQT among different treatment groups was performed. Tumor growth delay (TGD) is the difference of TVQTs of treated and untreated control tumors. When a treatment regimen proved to be effective, the study was extended up to 60 days. Animal body weights were measured 2 to 3 times a week as an indicator of apparent drug toxicity. Animals with a significant body weight loss (*e.g.* >15%) and/or signs of pain or distress were removed from the study and euthanized.

Therapeutic index (TI). TI is defined as the ratio of the maximum tolerated dose (MTD) to the minimum efficacious dose (MED). MTD is defined as the highest dose that results in no animal death or no more than 15% body weight loss. MED is defined as the lowest dose that results in tumor regression (*i.e.* tumor volume less than its initial treatment volume or equivalent to at least a minor response in clinic). Other tumor responses where the mean tumor volume is less than untreated control tumors and greater than its initial treatment volume is termed as tumor growth inhibition.

Table I. *Summary of therapeutic index of the treatment regimens used in this study.*

Tumor type	Drug	Dosing schedule	MTD (mg/kg)	MED (mg/kg)	TI
A375 melanoma	CKD-602	*qw* x 3, *i.v.*	>30	≤30	>1
		q4d x 4, *i.p.*	<20	≤3	~6
	Topotecan	*q4d* x 4, *i.p.*	8-20	3-8	≤2
	S-CKD602	*qw* x 3, *i.v.*	>1.5	0.15	>10
		q2w x 3, *i.v.*	~2.5	≤0.3	~8
		biw x 3, *i.v.*	1.0-1.5	0.1-0.3	~5
ES-2 ovary	CKD-602	*qw* x 3, *i.v.*	>30	≤30	>1
	S-CKD602	*qw* x 3, *i.v.*	~2.5	0.2-0.4	~6
H82 SCLC	CKD-602	*qw* x 3-4, *i.v.*	>30	>30	~1
	S-CKD602	*qw* x 3, *i.v.*	~2.5	0.5-1.0	~3
HT-29 colon	CKD-602	*qw* x 3, *i.v.*	>30	>30	~1
		q4d x 4, *i.p.*	<20	>20	<1
	Topotecan	*q4d* x 4, *i.p.*	<20	>20	<1
	S-CKD602	*qw* x 3, *i.v.*	~2.5	~0.75	~3

i.v.: intravenously; *i.p.*: intraperitoneally; MTD: maximum tolerated dose; MED: minimum efficacious dose; TI: therapeutic index (MTD/MED).

Results

A375 human melanoma xenografts (Tables I and II). Administering free CKD-602 *i.v.* weekly resulted in tumor growth inhibition at doses up to 20 mg/kg and tumor regression at 30 mg/kg with no apparent toxicity. TI was estimated to be >1. When free CKD-602 was given *i.p.* on the *q4d* schedule, tumors regressed at a dose as low as 3 mg/kg, and a 10% lethal dose (LD_{10}) was 20 mg/kg (TI ~6). Treatment with topotecan on the same *q4d* schedule had tumor regression at 8 mg/kg and an LD_{60} at 20 mg/kg (TI ≤2).

Following weekly administration of S-CKD602, tumor regression was observed at doses as low as 0.15 mg/kg. Complete tumor regression (CR) was observed at doses ≥0.3 mg/kg. No body weight loss was noted at doses up to 1.5 mg/kg. TI for S-CKD602 on the *qw* schedule was estimated to be >10. Administering S-CKD602 *q2w* yielded an MED at 0.3 mg/kg and an MTD at ~2.5 mg/kg (TI ~8). Giving S-CKD602 on the *biw* schedule resulted in an MED between 0.1 and 0.3 mg/kg and an MTD between 1.0 and 1.5 mg/kg (TI ~5).

These results indicate A375 was very sensitive to CKD-602, and S-CKD602 further enhanced the therapeutic efficacy over free CKD-602 and topotecan. Further, *qw* treatment with S-CKD602 resulted in a higher TI than the *q2w* schedule, and both schedules were superior to the *biw* schedule.

ES-2 human ovarian xenografts (Tables I and III). Weekly administration of free CKD-602 resulted in tumor regression at 30 mg/kg with no apparent toxicity. The TI for free CKD-602 was estimated to be >1. Weekly administration of S-CKD602 resulted in tumor regression

Table II. *Antitumor activity in the A375 human melanoma xenograft model.*

Drug	Dose (mg/kg)	Dosing[a] regimen	4x Growth delay (days)	No.	Max. tumor response	Max. BW loss	No. deaths	No. cures
CKD-602	10-20	*qw* x 3, *i.v.*	11.3-12.5	20	inhibition	-[b]	-	-
	30		>24.3	10	regression	-	-	-
CKD-602	3-8	*q4d* x 4, *i.p.*	13.6-16.0	20	regression	<2%	-	-
	20		>34.0	10	regression	>8%	1/10	-
Topotecan	3	*q4d* x 4, *i.p.*	8.9	10	inhibition	-	-	-
	8		18.2	10	regression	1%	-	-
	20		>26.2	10	regression	>16%	6/10	-
S-CKD602	0.1	*qw* x 3, *i.v.*	6.1	10	inhibition	-	-	-
	0.15		10.2	10	regression	-	-	-
	0.3		>24.9	10	regression	-	-	1/10
	0.5-1.0		>32.5-32.8	40	regression	1%	-	5/40
	1.5		>35.4	10	regression	1%	-	-
S-CKD602	0.3	*q2w* x 3, *i.v.*	8.9	10	regression	-	-	0
	0.6		20.7	10	regression	-	-	0
	2.5		>49.1	10	regression	19%	-	2/10
	3.0		>50.4	10	regression	24%	2/10	-
S-CKD602	0.1	*biw* x 3, *i.v.*	4.7	10	inhibition	-	-	-
	0.3		>38.8	10	regression	-	-	-
	1.0		>53.7	10	regression	5%	-	-
	1.5		-	10	regression	20%	10/10	-

[a] *qw* x 3: once a week for 3 weeks; *q4d* x 4: once every 4 days for 4 treatments; *q2w* x 3: once every 2 weeks for 3 treatments; *biw* x 3: twice a week for 3 weeks. [b] '-': none.

Table III. *Antitumor activity in the ES-2 human ovarian xenograft model.*

Drug	Dose (mg/kg)	Dosing[a] regimen	4x Growth delay (days)	No.	Max. tumor response	Max. BW loss	No. deaths	No. cures
CKD-602	30	*qw* x 3, *i.v.*	>9.4-27.5	21	regression	-[b]	-	1/9
S-CKD602	0.2	*qw* x 3, *i.v.*	2.8	8	inhibition	1%	-	-
	0.4		22.6	5	regression	-	-	-
	0.6		>27.4	7	regression	1%	-	4/7
	~0.75		>31.4	17	regression	4%	-	16/17
	1.0		>33.1	12	regression	-	-	-
	1.5		>35.6	10	regression	1%	-	10/10
	2.25		>60	12	regression	2%	-	8/12
	3.5		>60	12	regression	>6%	2/12	6/10

[a] *qw* x 3: once a week for 3 weeks. [b] '-': none.

at doses between 0.2 and 0.4 mg/kg. More than half of the treated animals achieved CR at 0.6 mg/kg, and more than 90% at 0.75-0.8 mg/kg. A slight weight loss (2%) was observed at 2.25 mg/kg, but 2/12 animal deaths occurred at 3.5 mg/kg. The TI for S-CKD602 was estimated to be ~6.

H82 human SCLC xenografts (Tables I and IV). In this model, tumor growth was inhibited (no regression) following free CKD-602 *qw* at doses up to 30 mg/kg. The TI for free CKD-602 was estimated to be >1. Weekly administration of S-CKD602 resulted in tumor growth inhibition at doses up to 0.5 mg/kg, and there was CR in more than 70% of treated

Table IV. *Antitumor activity in the H82 human SCLC xenograft model.*

Drug	Dose (mg/kg)	Dosing[a] regimen	4x Growth delay (days)	No.	Max. tumor response	Max. BW loss	No. deaths	No. cures
CKD-602	30	*qw* x 3, *i.v.*	>25.6	7	inhibition	7%	-[b]	2/7
S-CKD602	0.2	*qw* x 3, *i.v.*	2.4	7	inhibition	-	-	-
	0.5		>22.9	7	inhibition	-	-	1/7
	1.0		>33.9	7	regression	-	-	5/7
	3.0	*qw* x 4 i.v.	>40.5	12	regression	5%	-	11/12

[a] *qw* x 3 or 4: once a week for 3 or 4 weeks. [b] '-': none.

Table V. *Antitumor activity in the HT-29 human colon xenograft model.*

Drug	Dose (mg/kg)	Dosing[a] regimen	4x Growth delay (days)	No.	Max. tumor response	Max. BW loss	No. deaths	No. cures
CKD-602	15-35	*qw* x 3, *i.v.*	>6.5-10.4	48	inhibition	<7%	-[b]	-
CKD-602	3-8	*q4d* x 4, *i.p.*	5.3-6.5	10	inhibition	<7%	-	-
	20		16.0	10	inhibition	16%	4/10	-
Topotecan	3	*q4d* x 4, *i.p.*	5.2	10	inhibition	3%	-	-
	8		8.7	10	inhibition	9%	-	-
	20		-	10	inhibition	>21%	7/10	-
S-CKD602	0.3	*qw* x 3, *i.v.*	4.1	10	inhibition	2%	-	-
	0.75		>20.8	21	regression	6%	-	-
	1.5		>34.0	31	regression	6%	-	4/31
	2.5		>32.2	22	regression	21%	-	1/22
	3.0		>42.3	12	regression	>5%	2/12	-
	3.5		>49.6	12	regression	>6%	1/12	1/12

[a] *qw* x 3: once a week for 3 weeks; *q4d* x 4: once every 4 days for 4 treatments. [b] '-': none.

animals at a dose of 1 mg/kg or higher. A moderate weight loss (5%) was observed at 3 mg/kg. The TI for S-CKD602 was estimated to be >3.

HT-29 human colon xenograft model (Tables I and V). Weekly administration of free CKD-602 up to 35 mg/kg resulted in tumor growth inhibition and a 7% weight loss. Both the MTD and the MED for CKD-602 were greater than 35 mg/kg. Free CKD-602 was also compared to topotecan, both given *i.p.* on the *q4d* schedule. No tumor regression and only tumor growth inhibition was observed for both drugs up to 20 mg/kg, which represented an LD_{40} for the free CKD-602 and an LD_{70} for the topotecan. The TIs for the free drugs were both <1.

Weekly treatment of S-CKD602 was evaluated in a dose range from 0.3 to 3.5 mg/kg. Tumor regression was noted at 0.75 mg/kg, and there was CR at 1.5 mg/kg or higher. Significant weight loss was observed at ~2.5 mg/kg, and animal deaths occurred at 3.0 mg/kg or higher. The resulting TI for S-CKD602 was ~3.

Discussion

There is a large body of work describing the efficacy and safety of various treatment regimens of camptothecin compounds (7-12). For free CKD-602, the optimal dosing schedule in animal models has been shown to be once every 4 days, which produces antitumor activity superior to that of topotecan (2). CKD-602 has been approved for human use in South Korea for treatment of relapsed ovarian cancer and is a first-line agent for SCLC. The recommended treatment schedule for both indications is once daily for 5 days in a 21-day cycle.

S-CKD602 uses the STEALTH® liposomal delivery system to evade immune system interactions and prolong circulation of active drug. Liposomal formulations penetrate microvascular blood vessels and accumulate in the interstitial space of tumor tissue through defects in tumor vasculature formed during the angiogenesis process, whereas minimal drug accumulation occurs in postcapillary

and collecting venules in normal subcutaneous tissue (4-6). The prolonged circulation time and increased tumor tissue accumulation are expected to improve therapeutic outcome. S-CKD602 has demonstrated antitumor activity in many human tumor xenografts including ovarian, SCLC, melanoma, colon, breast, pancreas, prostate, and non-small cell lung cancers.

To properly evaluate S-CKD602, tumor regression was used as the MED criteria, which is more stringent than using tumor growth inhibition. Also, TI was derived for each test formulations to estimate their efficacy and safety margins. This approach proved to be tedious and time consuming since data need to be generated for different dose levels and dosing schedules. Despite of a total 14 separated studies in 4 tumor types conducted, we have observed consistently that tumor growth delay after S-CKD602 at ≥1 mg/kg doses was significantly greater than those after free CKD-602 at 30 mg/kg ($p<0.05$). For S-CKD602, we often observed the positive activity and dose relationship up to 1 mg/kg and little or no difference was noted at S-CKD602 doses of 1 mg/kg or higher.

Using the *qw* schedule, free CKD-602 at doses up to 35 mg/kg resulted in tumor regression in A375 and ES-2 models and only tumor growth inhibition in H82 and HT-29 models. Following treatment with S-CKD602 in A375, tumor regression was observed at doses as low as 0.15 mg/kg and CR occurred at a dose of 0.3 mg/kg or higher. In ES-2, tumor regression was seen with an S-CKD602 dose of 0.4 mg/kg; however, CR rate was >90% with higher doses. In H82, tumor regression occurred at a dose between 0.5 and 1.0 mg/kg, and CR rate was >50% at a dose of 1 mg/kg or higher. HT-29 was the least sensitive model among the four with tumor regression observed at 0.75 mg/kg or higher. The rankings of TIs (from high to low) for S-CKD602 in the 4 models studied were A375, ES-2, H82 and HT-29. On the other hand, the rankings of CR rates (from high to low) were ES-2, H82, A375 and HT-29.

Antitumor activity of S-CKD602 was compared to free CKD-602 and topotecan in A375 and HT-29 xenograft models. Tumor regression was observed in A375 but not in HT-29 tumors. The resulting TI for S-CKD602 was approximately 2-fold greater than that of free CKD-602 and 5-fold greater than topotecan in A375 tumors; it was at least 3-fold greater than both free drugs in HT-29 tumors.

The influence of the S-CKD602 dosing schedule on TI was also examined in A375 xenograft model. Three schedules (*i.e. biw*, *qw*, *q2w*) were compared. The TI was highest on the qw schedule (>10), followed by the *q2w* schedule (~8) and the *biw* schedule (~5). The optimal treatment schedule for safe and effective administration of S-CKD602 is once weekly in the A375 model.

Conclusion

We demonstrated the antitumor activity of S-CKD602 in a variety of human tumor types. When compared to free CKD-602 and topotecan, S-CKD602 provided a higher therapeutic index. Treatment schedule influenced the therapeutic index of S-CKD602; in the A375 human xenograft model in mice, a weekly dosing schedule was optimal.

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