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Antitumor Activity of 7-[2-(N-Isopropylamino)ethyl]-(20*S*)-camptothecin, CKD602, as a Potent DNA Topoisomerase I Inhibitor

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We developed a novel water-soluble camptothecin analogue, CKD602, and evaluated the inhibition of topoisomerase I and the antitumor activities against mammalian tumor cells and human tumor xenografts. CKD602 was a nanomolar inhibitor of the topoisomerase I enzyme in the cleavable complex assay. CKD602 was found to be 3 times and slightly more potent than topotecan and camptothecin as inhibitors of topoisomerase, respectively. In tumor cell cytotoxicity, CKD602 was more potent than topotecan in 14 out of 26 human cancer cell lines tested, while it was comparable to camptothecin. CKD602 was tested for the *in vivo* antitumor activity against the human tumor xenograft models. CKD602 was able to induce regression of established HT-29, WIDR and CX-1 colon tumors, LX-1 lung tumor, MX-1 breast tumor and SKOV-3 ovarian tumor as much as 80, 94, 76, 67, 87% and 88%, respectively, with comparable body weight changes to those of topotecan. Also the therapeutic margin (R/Emax: maximum tolerance dose/ED_{58}) of CKD602 was significantly higher than that of topotecan by 4 times. Efficacy was determined at the maximal tolerated dose levels using schedule dependent i.p. administration in mice bearing L1210 leukemia. On a Q4dx4 (every 4 day for 4 doses) schedule, the maximum tolerated dose (MTD) was 25 mg/kg per administration, which caused great weight loss and lethality in <5% tumor bearing mouse. This schedule brought significant increase in life span (ILS), 212%, with 33% of long-term survivals. The ex vivo antitumor activity of CKD602 was compared with that of topotecan and the mean antitumor index (ATI) values recorded for CKD602 were significantly higher than that noted for topotecan. From these results, CKD602 warrants further clinical investigations as a potent inhibitor of topoisomerase I.

Key words : Camptothecin, CKD602, Topoisomerase I, Antitumor, Xenograft, *Ex vivo* pharmacodyanmics

INTRODUCTION

Camptothecin (CPT) is a plant antitumor alkaloid isolated from *Camptotheca acuminata* (Wall *et al.*, 1966). Although CPT showed a good *in vitro* cytotoxicity, its development was halted because of severe toxicities (Creaven *et al.*, 1972; Gottlieb *et al.*, 1972; Moertel *et al.*, 1972; Muggia *et al.*, 1972) including myelosuppression and hemorrhagic enterocolitis in clinical trials. It was considered that poor aqueous solubility of the drug was responsible for the toxicities in part. But it was recently demonstrated that CPT had a unique mechanism of action, inhibition of DNA topoisomerase type I (topo I) (Hsiang and Liu, 1988; Hsiang *et al.*, 1985), compared with many other anticancer drugs affecting the activity of DNA topoisomerase type II (topo II) (Hsiang *et al.*, 1988; Rose, 1988). These enzymes can relieve the torsional strain associated with replication, but that topo I normally performs this function. In contrast to topo II, the levels of topo I do not appear to be closely linked to proliferation in normal tissues. Furthermore, topo I level in several neoplastic cell types, including carcinomas of the colon, ovary and esophagus, as well as lymphomas, may be higher than in adjacent normal tissues (Bodley and Liu, 1988; Giovanelta *et al.*, 1989). Since cells

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with higher levels of topo I are more sensitive to topo I-targeting agents, the elevated levels of the enzyme in tumor cells might be of therapeutic importance. For that reason, the development of camptothecin-like compounds as inhibitors of topoisomerase I for the treatment of resistant tumors has generated clinical excitement in this class of drugs. Earlier studies with soluble sodium salt of the open-ring hydroxy camptothecin resulted in unacceptable toxicities in human, including severe hemorrhagic cystitis and myelosuppression which limited further development (Muggia *et al.*, 1972; Gottlieb *et al.*, 1970). After CPT's mechanism of action was recognized, structure-activity studies were performed to focus on two portions of the CPT molecule, E-ring, A-ring and B-ring (Fig. 1) (Kingsbury *et al.*, 1989). E ring lactone and the alpha hydroxyl group at position 20 are essential to stabilize topo-I-DNA adducts. Modifications on the A and B rings resulted in CPT analogues with increased water solubility and activity. These are being clinically used, topotecan and irinotecan. We synthesized an analogue of camptothecin, CKD602, in which water-solubilizing group was introduced at position 7 of the B-ring (Jew *et al.*, 1995; Jew *et al.*, 1996a; Jew *et al.*, 1996b; Lee *et al.*, 1998). This compound was evaluated in several *in vitro* assays including a cell cytotoxicity and a cleavable complex assay as well as antitumor activity and efficacy depending on the schedule of administration using HT-29, WIDR and CX-1 colon tumors, LX-1 lung tumor, MX-1 breast tumor, SKOV-3 ovarian tumor xenograft and L1210 leukemia bearing model. In addition, we compared the antitumor activity of CKD602 with that of topotecan by determining the *in vitro* inhibitory activity of the beagle dogs' ultrafiltrable plasma against 12 human tumor cell lines on the basis of *ex vivo* pharmacodynamics.

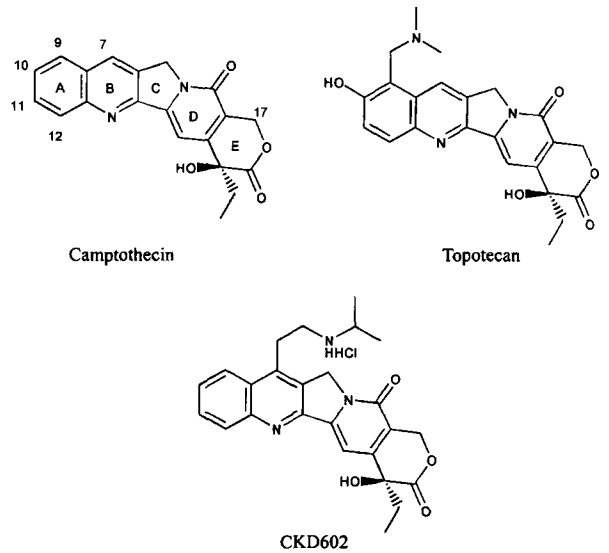

Fig. 1. Chemical structures of CKD602, topotecan, and camptothecin.

MATERIALS AND METHODS

Test compounds

CKD602 and topotecan (Fig. 1) were semi-synthesized by the previous reported methods (Patent, publication number: WO96-21666). The drugs were formulated just before the injection on each day of treatment and was dissolved and diluted to its final concentrations in 10% PEG400 adjusted pH to 3.0.

Animals

Male athymic nude mice (BALB/c-nu/nu) aged 4 weeks were purchased from Charles River Japan. They were housed in an exclusive experimental room and were given sterilized food and water ad libitum. Male BDF1 mice aged 4 weeks were purchased from Charles River Japan. They were housed in controlled temperature, 23±0.5°C. Male Beagle dogs weighing 10~15 kg were purchased from The Jackson Laboratory (USA).

Tumors

Poorly differentiated mammary carcinoma MX-1, poorly differentiated colon adenocarcinomas HT-29, CX-1 and WIDR, poorly differentiated lung carcinoma LX-1, and poorly differentiated ovarian carcinoma SKOV-3 were graciously provided by DKFZ TumorBank. L 1210 lymphocytic leukemia was obtained from KCLB, Seoul, Korea.

Topoisomerase inhibition assay

The ability of camptothecin analogues to inhibit topoisomerase I was quantified in the cleavable complex assay as described previously (Tewey *et al.*, 1984; Hsiang *et al.*, 1985; Liu *et al.*, 1983).

Evaluation of cytotoxic activity

The *in vitro* antitumor activity of the drugs was evaluated against a panel of human cancer cell lines by tetrazolium-dye (MTT) assay. In brief, single cell suspensions were prepared by mechanical disaggregation or treatment with trypsin and ethylenediamine tetraacetic acid (EDTA) followed by mechanical disaggregation. Seeding numbers and incubation periods were determined after confirmation of the linear relationship between the absorbance (optical density, OD) and number of cells plated in standard and the growth curve generated for each cell line. After the viability was confirmed as being over 95% by trypan blue dye exclusion, cells were counted using a hemocytometer, diluted with RPMI-fetal bovine serum (FBS), and plated in 96-well plates at final concentrations (180

μl/well) of number of cells determined as above. Cells were then treated with 20 μl of drug solutions. For each drug, eight concentrations were used, spanning a range of 3~5 log concentrations. After the incubation for 4 days at 37°C in a humidified incubator containing 5% CO_2, 50 μl of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) dissolved in phosphate-buffered saline (2.5 mg/ml) was added to each well and the plates were incubated for an additional 4 h. To solubilize the formazan crystals formed, 200 μl of dimethylsulfoxide (DMSO) was added to each well and the plates were shaked by plate shaker (Dynatech, minishaker, USA), for about 5 min, resulting in good solubilization. The OD was measured at 540 nm using an enzyme-linked immunosorbent assay (ELISA) reader (BIO-TEK, ELx808, USA). Each experiment was performed three times and the mean absorbance values for each drug concentration were calculated. The IC_{50} value was defined as the drug concentration that produced a 50% reduction of absorbance at 540 nm in drug treated cells as compared with untreated controls.

Evaluation of antitumor activity

Solid tumors, CX-1, HT-29, WIDR, LX-1, MX-1, and SKOV-3 were maintained by serial subcutaneous transplantation in athymic nude mice. They were harvested aseptically from donor mice, and then skin, connecting tissue, and necrotic parts were removed. Prepared tumor mass of 50 mg were implanted into the right axillary region by 16-gauge trocar. Experiments started when the tumor size was 100~200 mm^3 (day1). Tumor volume was calculated from two-dimensional measurements using the following equation:

$$\text{Tumor volume } (mm^3) = (\text{length} \times \text{width}^2) \times 0.5$$

L1210 lymphocytic leukemia was maintained by serial intraperitoneal transplantation in BDF1 mice. L 1210 tumor cells were harvested aseptically from the peritoneal cavity of donor mice with a 23-gauge needle, pooled, and diluted with cold saline. The cell suspensions were adjusted to 1×10^7 cells/ml. An inoculum of 0.1 ml of L1210 was given by intraperitoneal implantation into BDF1 mouse using a 25-gauge needle. At 24 hrs after tumor implantation, drug treatment started (day1).

Efficacy was assessed by prolongation of mean survival time (ILS), or in the case of solid tumors, inhibition of tumor growth (IR) as determined by mean tumor volume and weight (IRtw) on the day 21 post-treatment.

Ex vivo pharmacodynamics study

Drug administration: The drugs were dissolved in water for injection, containing d-mannitol 50 mg/ml and tartaric acid 0.06 mg/ml, just before the experiments. A single dose of 1 mg/kg and 0.74 mg/kg of CKD602 and topotecan, respectively, was given as a 30-min infusion to each dog in a volume of 3 ml/kg.

Blood-sample preparation: Blood was sampled from beagle dogs through an i.v. cannula placed in the cephalic vein into a heparin-containing syringe prior to the drug administration and at 0, 10, 30, 60, 120 and 240 minutes after the end of infusion. Plasma was immediately separated by centrifugation at 600 g for 10 min. As soon as plasma had been prepared, a part of it was passed through an Amicon CF 4104 filter (Amicon Corp., USA) by centrifugation at 1500 g for 20 min at 4°C to remove protein. The protein-free ultrafiltrates for bioassay were stored at -70°C until analysis.

Antitumor activity test based on *ex vivo* pharmacodynamics: The *in vitro* antitumor activity of the ultrafiltrable plasma obtained from beagle dogs treated with equitoxic doses of the two drugs was evaluated against 12 human cancer cell lines by tetrazolium-dye assay (see **Evaluation of cytotoxic activity** for details). Three human colon carcinoma cell lines (CX-1, HT-29 and WIDR), three human ovarian carcinoma cell lines (CAOV-3, OVCAR-3 and SKOV-3), two human lung carcinoma cell lines (LX-1 and A549), two human breast carcinoma cell lines (MX-1 and MCF-7) and two human liver carcinoma cell lines (HepG2 and Hep3B) were used. The biological antitumor activity was determined in terms of ATI, which was defined as the area under the inhibition rate versus time curve plotted from 0 to 240 min by bioassay and calculated by the trapezoidal rule.

RESULTS

Topoisomerase I inhibition and tumor cell cytotoxicity

The ability of CKD602 to inhibit human DNA topoisomerase I was determined in the cleavable complex assay as demonstrated in Fig. 2. All three compounds tested were demonstrated to be the potent inhibitors of topoisomerase I. CKD602 was found to be 3 times and slightly more potent than topotecan and camptothecin, respectively, as determined by their IC_{50}s. The cytotoxicity toward cultured human tumor cell lines was determined using the MTT microculture assay, and reported in table I. The results represent mean IC_{50} values for three experiments. In 14 out of 26 human cancer cell lines, the cytotoxicity of CKD 602 was 1.4-10 times as potent as that of topotecan. In the 7 cell lines, CKD602 showed lower cytotoxicity than topotecan (potency ratio < 0.84) did. The other 5 cell lines showed comparable cytotoxicity to topotecan (potency ratio 1-1.1). The differences in IC_{50} values were most striking in the case of SNU-1, followed

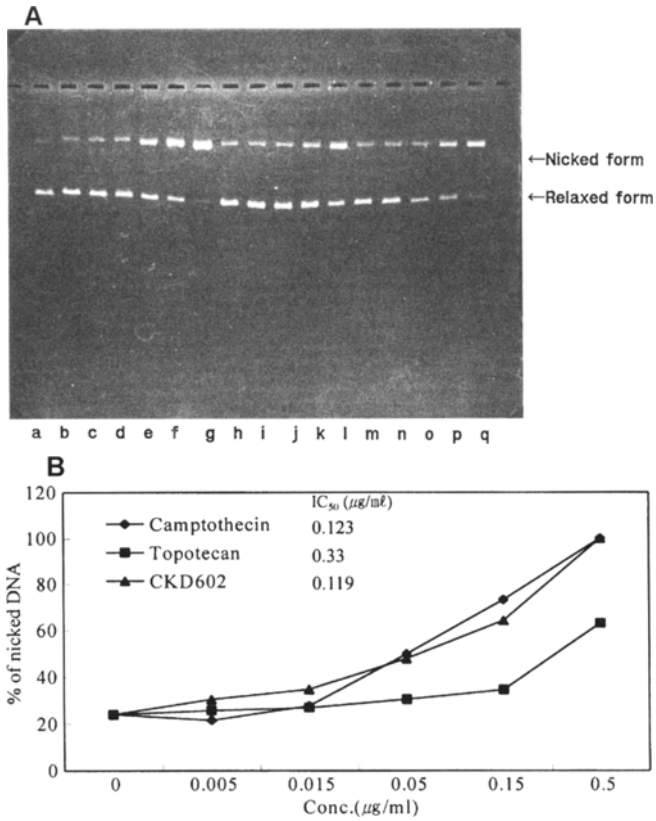

Fig. 2. Comparison of topoisomerase I-mediated DNA cleavage induced by camptothecin, topotecan, and CKD602. Supercoiled pBR 322 (0.25 μg) was incubated with 4 units of human topoisomerase I in the presence or absence of drugs followed by SDS/proteinase K treatment and then analyzed on an agarose gel containing 0.5 μg/ml EtBr. Lane a, substrate pBR 322; b, no drug; c~g, camptothecin; h~l, topotecan; m~q, CKD 602. Drug concentrations were: lane c, h, and m, 0.005 μg/ml; d, i, and n, 0.015 μg/ml; e, j, and o, 0.05 μg/ml; f, k, and p, 0.15 μg/ml; g, l, and q, 0.5 μg/ml.

by A549 (potency ratios 10 and 8.33, respectively). However, all three topoisomerase I inhibitors demonstrated little susceptibility to multidrug resistance as determined by comparing the IC_{50}s obtained using high levels of *mdr* 1 gene expression cell lines (SNU-354 and SNU-368) to those observed with low levels of *mdr* 1 gene expression cell line (SNU-182). The SNU-368/SNU-182 IC_{50} ratios were determined to be >35 for CKD602, >12 for topotecan and >35 for camptothecin.

Antitumor activity in xenograft models

The ability of CKD602 to effect the growth of the HT-29, WIDR and CX-1 human colon tumors, LX-1 human lung, MX-1 human breast and SKOV-3 human ovarian tumor xenografts was assessed by monitoring tumor growth kinetics over a 3-weeks dosing period. The body weight and tumor size were determined for each group on every 4 day, and drugs were dosed every 4 day for 4 doses (Q4dx4). To ensure an equal comparison, the compounds were dosed to the limit of toxicity as determined by maximal IR (IRmax) value loss appearing. Table II and III summarized the efficacy of CKD602 and topotecan scheduled by Q4dx4, i.p. injection, respectively. At total dose of 80 or 100 mg/kg, CKD602 was significantly effective against HT-29, WIDR, CX-1, LX-1, MX-1, and SKOV-3 yielding IRtw values of 79.8, 93.6, 75.8, 66.7, 87% and 88.6%, respectively. CKD602 significantly inhibited the growth of WIDR at doses of 30~80 mg/kg, and the therapeutic margin, R/Emax, was significantly higher, 4-times as high as than that of topotecan. However, the loss of body weight was increased, according to the dose dependency of CKD602 in all tested tumors. Especially, in WIDR colon xenograft it showed the highest activity at 80 mg/kg but demonstrated chronic toxicity, resulting in a body weight loss of 17.4% on the day 17 which was comparable to that of topotecan. In all tests mentioned above, the IRmax values and R/Emax value for mice receiving CKD602 were higher than those for animals given topotecan. These data coincide with the relative differences seen in potency in the cleavable complex and the MTT assay.

Antitumor activity on dose schedule difference

On the Q4dx4 dosing schedule, CKD602 showed strong antitumor activity in all tested xenograft models. But the dosing schedule may bring the difference in vivo efficacy of CKD602 and topotecan. Table IV demonstrated the efficacy of CKD602 determined at maximal tolerated dose levels using the schedule dependent on i.p. administration in mice bearing L1210 leukemia. In the case of CKD602, the 5-daily injection showed a higher ILS of 192% than 164% ILS of the single bolus injection at the dose of 30 mg/kg. Furthermore, intermittent treatments of CKD602, Q 4dx4, Q4dx2 and Q7dx2, resulted in 212, 208% and 186% ILS, respectively. Of these dosing schedules, Q 4dx4 injection prolonged life span by 118~212%, which was dependent on the dose levels, ranged 15~100 mg/kg and showed mean survival time of 30 days and long-term survivals of 2 mice. In contrast, topotecan, administered on the single or 5-daily schedule, had comparable increases in life span and greater activity was seen when the intermittent treatment was used (Table V). However, unlike CKD602, the Q4dx2 injection showed more increase in life span than the Q4dx4 or Q7dx2 injection. In all tests mentioned above, the results indicated that CKD602 administered on the intermittent dosing schedules was more efficacious than single bolus injection.

Comparison of antitumor activity based on *ex vivo* pharmacodynamics

The antitumor activity of CKD602 was compared

Table I. Comparison of the *in vitro* antitumor activity of camptothecin, topotecan and CKD602 in several murine and human cancer cell lines

Cell lines	*Mdr 1* Expression[b]	IC_{50} (ng/ml)			Potency ratio[a]
		Camptothecin	Topotecan (A)	CKD602 (B)	A/B
Murine origin					
B16	NT	21.5	190	36.6	5.19
3LL	NT	22.9	10.3	6.63	1.55
Colon 26	NT	6.56	20.7	22.9	0.90
L1210	NT	18.3	102	222	0.46
P388	NT	216	448	1001	0.45
EL-4	NT	1.72	13.9	5.86	2.37
Human origin					
Glioblastoma:					
U87	NT	0.171[d]		0.05[d]	
SW1783	NT	0.049[d]		0.026[d]	
Stomach:					
KATO-III	NT	16	420	160	2.63
SNU-1[c]	L	4.3	24	2.5	10
SNU-5[c]	L	1.8	17	63	0.29
SNU-16[c]	L	9.4	3	2.7	1.11
Colon:					
DLD-1	NT	4.8	240	170	1.41
HT-29	NT	6.74	28.4	10.9	2.61
WIDR	NT	13.1	67.6	27.6	2.45
SNU-C1[c]	L	13	21	4.3	5.12
SNU-C2A[c]	L	>3000	>3000	>3000	1
SNU-C4[c]	H	31.7	41	61	0.68
Liver:					
SNU-182[c]	L	84	238	84	2.82
SNU-354[c]	H	>3000	>3000	>3000	1
SNU-368[c]	H	>3000	>3000	>3000	1
Lung:					
A549	NT	0.113	75	9.0	8.33
NCI-H358	NT	47	301	2053	0.15
NCI-H522	NT	145	718	90	7.98
NCI-H1299	NT	297	1152	573	2.01
Breast:					
MCF-7	NT	1967	2066	2476	0.83
MDA-MB-231	NT	228	539	345	1.57
ZR-75-1	NT	51	235	290	0.81
Ovary:					
CAOV-3	NT	1.02	1.77	10.2	0.17
SKOV-3	NT	13	31	31	1
OVCAR-3	NT	1419	1762	1249	1.43
Melanoma:					
SK-MEL-1	NT	215	1631	1889	0.84
SK-MEL-2	NT	72	289	82	3.52
SK-MEL-3	NT	98	597	78	7.65

[a]Ratio of IC_{50} values for the two drugs (topotecan/CKD602).
[b]Determined by slot-blot analysis of mRNA: L low (below 5U), I intermediate (5-30U), H high (above 30U) levels of *mdr 1* mRNA expression.
[c]Clinically isolated cell lines in Seoul Natl. Univ. Cancer Research Center, Seoul, Korea.
[d]μM.
NT: not tested.

with that of topotecan using ATI determined from *ex vivo* pharmacodynamics results of IR versus time curves (Table VI). Fig. 3 shows IRs found for CKD602 and topotecan in plasma from 0 to 240 min after drug administration. The IR of topotecan decreased rapidly as compared with that of CKD602; consequently, the antitumor activity of topotecan was not detected after 240 min against all 12 cancer cell lines. The mean ATI values noted for CKD602 were significantly higher than those obtained for topotecan, and were shown to be ranked in the following order: ovarian carcinoma > lung carcinoma > colon carcinoma > liver carcinoma >

Table II. Antitumor activity of CKD602 in human tumors bearing athymic nude mice

Tumor	R/Emax[b]	Total dose[d] (mg/kg)	IR%[a]	BW loss (%)[c]
CX-1	3.48	30	44.6	
		60	64.2	5.8
		80	68.9	10.8
		100	75.8	17.5
HT-29		15	58.3	9.9
		30	65.5	6.2
		60	79.8	10.3
WIDR	6.68	15	45.1	8.7
		30	70.3	12.4
		60	90.8	11.0
		80	93.6	17.4
LX-1	2.17	15	53.3	1.9
		60	64	14.5
		100	66.7	14.1
MX-1	3.8	15	39.7	2.0
		60	76.5	9.8
		100	87	35.2
SKOV-3	3.8	15	40.8	14.2
		60	88.0	24.5
		100	88.6	

[a]inhibition rate (1-TWt/TWc)x100, TWt: the mean tumor weight of the treated group, TWc: the mean tumor weight of the control group.
[b]Therapeutic index (MTD/ED), MTD: maximum tolerated dose, ED: effective dose.
[c]Maximal body weight change, relative to the body weight of the day1.
[d]Total dose given for the treatment schedule, Q4dx4.

Table III. Antitumor activity of topotecan in human tumors bearing athymic nude mice

Tumor	R/Emax[b]	Total dose[d] (mg/kg)	IR%[a]	BW loss (%)[c]
CX-1	1.33	20	17.7	0.7
		40	25.8	0.9
		60	36.4	1.3
		80	41.3	4.7
HT-29		10	26.2	12.1
		20	7.1	12.0
		40	35.7	13.4
WIDR	1.46	10	35.0	4.0
		20	45.8	8.3
		40	44.8	14.9
		60	54.9	15.6
LX-1	1.03	20	38.7	17.7
		40	40	0.9
		80	58.7	27.5
MX-1	1.6	40	23.5	30.0
		80	80.3	10
		100	76.5	
SKOV-3	1.6	20	40.8	
		60	88.0	
		100	88.6	

[a]inhibition rate (1-TWt/TWc)x100, TWt: the mean tumor weight of the treated group, TWc: the mean tumor weight of the control group
[b]Therapeutic index (MTD/ED), MTD: maximum tolerated dose, ED: effective dose
[c]Maximal body weight change, relative to the body weight of the day1
[d]Total dose given for the treatment schedule, Q4dx4

Table IV. Schedule-dependent efficacy of CKD602 in BDF1 mice-bearing L1210 leukemia

Schedule	Total dose (mg/kg)	ILS%[a]	30-day survivals	BW loss[b]
Single	15	99.3		
	30	164.1	1	
	60	152.1	1	5.0
	80	90.9		3.8
5 daily	15	169.4	1	12.6
	30	191.7	1	26.4
Q4dx4	15	118.5		
	30	179.2		
	60	202.5	1	
	80	202.5	2	
	100	212.5	2	2.4
	120	181.8	2	19.0
Q4dx2	15	141.5		7.3
	30	170.4		
	60	208.1	1	0.3
	100	163		20.5
Q7dx2	15	126.7		
	30	162.5	1	
	60	186.4	1	
	100	162.5		16.0

[a]Increase in life span of mice bearing L1210 leukemia, relative to the controls (MSTt/MSTc-1)x100, MSTt: the mean survival time of the treated group, MSTc: the mean survival time of the control group.
[b]Maximal body weight change, relative to the body weight of the day1.

breast carcinoma.

DISCUSSION

The use of the mechanism oriented cleavable complex assay together with *in vitro* cell cytotoxicity assay led to the launch of irinotecan (Topotecin®) and topotecan (Hycamtin®) in the market. We have synthesized a novel analogue of camptothecin, CKD602, in which water-solubilizing group was introduced at position 7 of the B ring (Jew *et al.*, 1995; Jew *et al.*, 1996a; Jew *et al.*, 1996b; Lee *et al.*, 1998). The purpose of this study was to evaluate the topoisomerase I inhibitors in both *in vitro* enzyme assay and cytotoxicity assay and to compare its relative antitumor effectiveness in tumor xenograft models for further clinical trials. Since topotecan is an active topoisomerase I inhibitor and has active *in vivo* profiles, we utilized it as a reference drug for the comparison with our compound. CKD602 was found to be a more potent inhibitor in the cleavable complex assay and, demonstrated significantly greater potency in 4 of the 26 cell lines compared to topotecan when assessed in the MTT cell cytotoxicity assay. The present study demonstrated that i.p. administration of CKD602 was significantly effective against human tumor xenografts transplanted into nude mice. CKD602 injected i.p. every 4 day for a total of four treatments showed higher antitumor

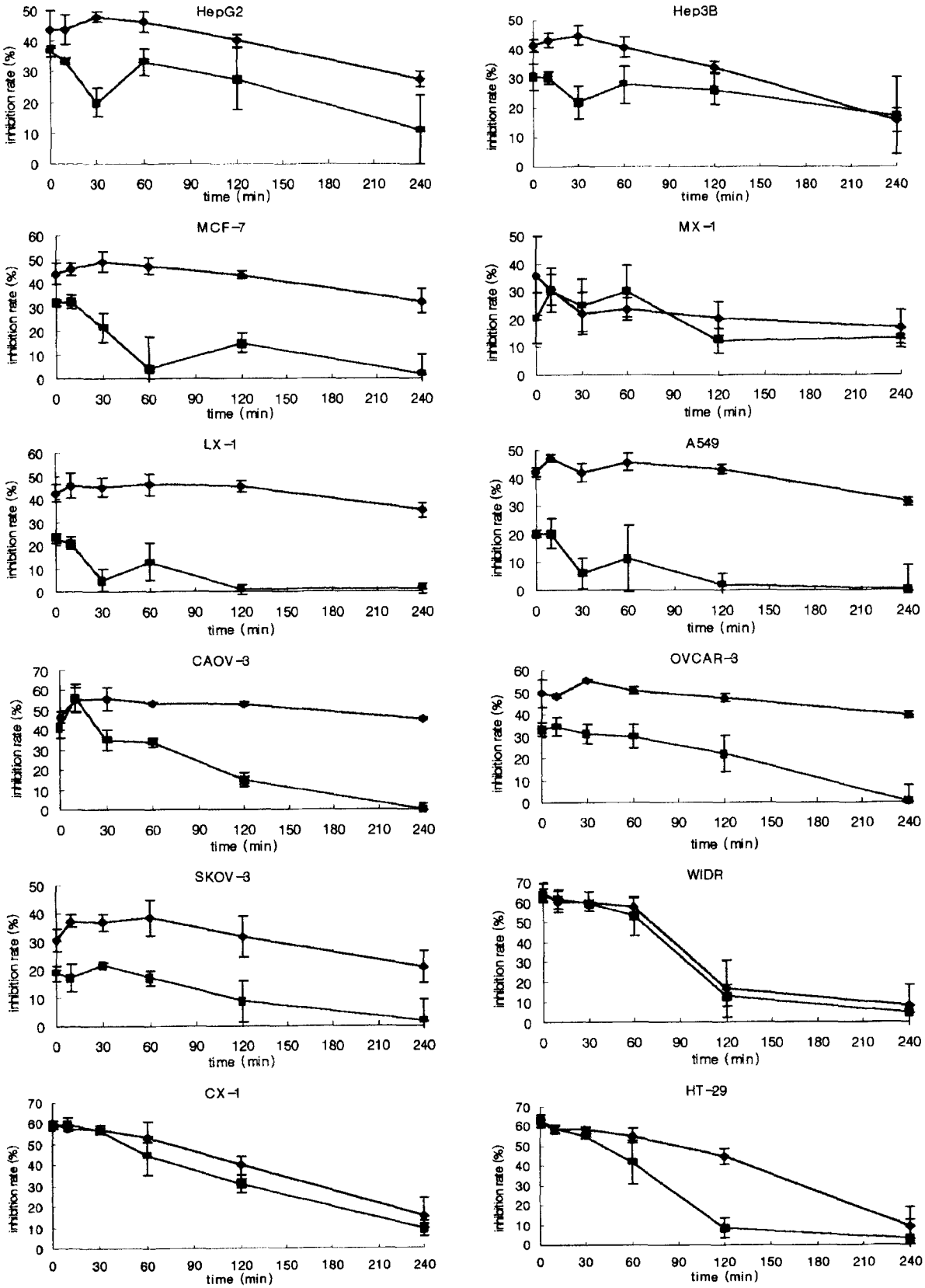

Fig. 3. Comparison of the antitumor activity of ultrafiltrable plasma obtained from beagle dogs treated with CKD602 (1 mg/kg, ◆) and topotecan (0.74 mg/kg, ■), respectively, as determined by ex vivo pharmacodynamic assay. Each point represents the mean value±SD for three independent experiments.

Table V. Schedule-dependent efficacy of topotecan in BDF 1mice-bearing L1210 leukemia

Schedule	Total dose (mg/kg)	ILS%[a]	30-day survivals	BW loss[b]
Single	10	89.7		5.2
	20	143.1		3.0
	40	152.1	1	
	60	93.9		
5 daily	10	147.2		1.8
	20	158.3	1	22.4
	40	158.3	1	23.9
	80	11.1		27.4
Q4dx4	10	65.3		
	20	135.3		
	40	176.6		
	60	179.2		
	80	192.1	2	
	100	176.1	1	
Q4dx2	10	66.7		9.0
	20	166.7		
	40	200	1	
	80	208.1	1	22.8
Q7dx2	10	126.7		
	20	150.6		
	40	165.5		
	80	165.5	1	3.4
	120	131.5		22.2

[a]Increase in life span of mice bearing L1210 leukemia, relative to the controls (MSTt/MSTc-1)x100, MSTt: the mean survival time of the treated group, MSTc: the mean survival time of the control group.
[b]Maximal body weight change, relative to the body weight of the day1.

Table VI. Comparison of the antitumor activity of CKD602 and topotecan against 12 human cancer cell lines by ATI[a]

Origin	Cell lines	ATI	
		CKD602	Topotecan
Colon	CX-1	9529±768.9	7496±529.2
	HT-29	9752±548.7	5473±471.1
	WIDR	7299±2099.4	6697±1397.4
	Mean±SD	8860±1644.5**	6555±1180.0
Ovary	CAOV-3	12350±181.7	4875±495.3
	OVCAR-3	11284±75.7	4968±1194.9
	SKOV-3	7449±1361.0	2928±1391.6
	Mean±SD	10361±2335.8**	4257±1377.6
Lung	LX-1	10288±696.7	2201±410.7
	A549	9792±266.4	2014±486.9
	Mean±SD	10040±544.3**	2107±415.6
Breast	MX-1	4357±1120.0	4261±456.5
	MCF-7	10141±664.4	3348±51.6
	Mean±SD	7249±3272.9*	3782±556.9
Liver	HepG2	9583±413.1	5852±1556.5
	Hep3B	7815±406.7	5872±1800.1
	Mean±SD	8699±1035.3**	5861±1505.0
Total	Mean±SD	9137±2186.2**	4662±1899.1

*Significantly different from the value obtained for topotecan ($P<0.05$).
**Significantly differently from the value obtained for topotecan ($P<0.01$).
[a]ATI was defined as the area under the %IR versus time curve plotted from 0 to 240 min as obtained by ex vivo pharmacodynamic assay and was calculated by the trapezoidal rule.

activity than topotecan against six human tumors included HT-29, WIDR and CX-1 colon adenocarcinoma, LX-1 lung adenocarcinoma, MX-1 mammary carcinoma and SKOV-3 ovarian tumor. These in vivo antitumor effects were well in accord with the result of the cleavable complex assay. Especially, more broad therapeutic margins will be effective against non small-cell lung cancer including squamous-cell lung carcinoma. However the body weight loss resulted from the drug treatment, similar to that from topotecan, was in a dose-dependent fashion. The maximum weight loss occurred at the maximum effective dose level and the weight loss was reversible upon the withdrawal of the drug. Those might be concerned with the delayed toxicity observed in acute and subacute toxicity study of CKD602 (KRICT report- Part I, 1998; KRICT report-Part II, 1998). Four (Q4dx4) i.p. injections of CKD602 gave higher values for ILS and smaller values for body weight loss than those obtained following a single i.p. injection at the same total dose of 80 mg/kg. These data mean that a schedule of intermittent injection is superior to a single injection in antitumor activity and safety. Thus, the effect of CKD602 depends on the schedule of administration. It is known that mammalian cells in the S-phase show the highest sensitivity to CPT (Drewinko *et al.*, 1974; Li *et al.*, 1972). Recent studies have been reported that the inhibition of type 1 DNA topoisomerase in S phase cells by CPT causes the arrest of replication forks and results in cell killing (Hsiang *et al.*, 1989). The S phase-specific effect of CPT may offer a possible reason for the schedule dependent on the antitumor activity of CKD602, as demonstrated in other S phase-specific drugs (Skipper *et al.*, 1970). In conclusion, CKD602 exhibits potent antitumor activity in the cleavable complex assay and the human tumor xenograft models. This compound is therefore expected to be clinically effective if its dose and the schedule of administration are suitably controlled.

For the evaluation of the antitumor activity of new compounds under investigational status, a number of *in vitro* and *in vivo* test systems have been developed. However, these evaluation systems have some limitations in predicting the clinical activity. When an antitumor drug is given to humans, the response to the drug is thought to be influenced by two major factors: one is the amount of the active form of the drug *in vivo,* which is usually determined by its concentration and duration, and the other is the sensitivity of tumor cells to the drug. We thought it is desirable to predict the clinical response of new antitumor compounds in the preclinical stage with reliable parameters. Therefore,

in this study the antitumor activity of CKD602 was investigated according to the concept described by Sasaki *et al.* and was compared with that of topotecan. CKD602 indicated significantly higher ATI than topotecan against all 12 human cancer cell lines tested. Furthermore, the mean ATI values of CKD602 was over two-fold higher than those of topotecan in breast, ovarian and lung carcinoma cell lines. Considering the previous report (Sasaki *et al.*, 1991) that the ATI values determined by *ex vivo* pharmacodynamics in humans showed a good correlation with the clinical response, the high mean ATI value recorded for CKD 602 in this study suggests that this drug would show encouraging antitumor activity in a clinical study. These results suggest that CKD602 warrants further clinical investigations as a potent inhibitor of topoisomerase I.

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