ANTITUMOR ACTIVITIES OF (2″*R*)-4′-*O*-TETRAHYDROPYRANYL-ADRIAMYCIN (THP) AND ITS COMBINATION WITH OTHER ANTITUMOR AGENTS ON MURINE TUMORS

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(2″*R*)-4′-*O*-Tetrahydropyranyladriamycin (THP) is a new derivative of doxorubicin (adriamycin, ADM). The concentrations of THP and ADM required to inhibit by 50% the growth of a cultured L1210 cells was 0.003 μg/ml and 0.016 μg/ml, respectively. Various therapeutic designs of combinations of THP with other antitumor agents were investigated *in vivo* using the L1210 murine leukemia. The combination of THP with cytosine arabinoside (Ara-C), cyclocytidine hydrochloride (Cyclo-C), 6-mercaptopurine (6-MP) and cyclophosphamide (EX) showed a great effectiveness following daily intraperitoneal treatment from days 1 to 10. High therapeutic effects were also obtained with the combinations of THP with Ara-C, Cyclo-C, vincristine (VCR) and EX following intravenous combination therapy one day following implantation of L1210 leukemia.

Schedule dependency and its therapeutic efficacy of THP were examined. THP showed almost the same antitumor activity on the solid-type sarcoma-180 or solid-type Ehrlich carcinoma as ADM by intraperitoneal or intravenous treatment. THP showed some superior activity to ADM in the advanced stage of L1210 leukemia. High antitumor activity of THP on murine leukemia L1210 has been reported by TSURUO *et al.* (Cancer Res. 42: 1462~1467, 1982) and was also confirmed. THP gave many mice cures, especially in the intravenous treatment.

(2″*R*)-4′-*O*-Tetrahydropyranyladriamycin (THP) is a new derivative of doxorubicin (adriamycin, ADM). The preparation and antitumor activity on some experimental tumors have been reported by UMEZAWA *et al.*[1] and TSURUO *et al.*[2]. THP had lower cardiac toxicity in hamsters than ADM and other anthracyclines[3]. Other studies on the toxicology, pharmacology and pharmacodynamics of this new antitumor drug have been performed. Some of them have been reported[4] and others are being prepared for publication. The clinical study of THP has also been initiated against various types of cancer[5,6].

In this paper, the cytotoxicity against L1210 leukemia *in vitro*, the antitumor activity against sarcoma-180 and Ehrlich carcinoma, and the schedule dependency of THP are described. The combination effects *in vivo* of THP with various other antitumor agents against L1210 leukemia are also reported.

Materials and Methods

Animals and Tumors

Male hybrid mice (BALB/c×DBA/2)F_1(CDF$_1$) were obtained from Shizuoka Agricultural

Cooperative Association for Laboratory Animals (Hamamatsu). ICR mice were from Charles River Japan Inc. (Atsugi).

Murine leukemia L1210, sarcoma-180 and Ehrlich carcinoma have been maintained by weekly transfer in our laboratory. Cultured L1210 cells have also been maintained by 3~4 days transfer in our laboratory.

Drugs

THP and aclarubicin (aclacinomycin, ACM) were obtained from Sanraku-Ocean Co., Ltd. (Tokyo). Daunorubicin (DM) was purchased from Meiji Seika Kaisha, Ltd. (Tokyo). Cytosine arabinoside (Ara-C) and cyclocytidine hydrochloride (Cyclo-C) were purchased from Nihon Shinyaku Co., Ltd. (Kyoto) and from Yamanouchi Pharmaceutical Co., Ltd. (Tokyo), respectively. 6-Mercaptopurine (6-MP), methotrexate (MTX) and chromomycin A3 (CHR) were purchased from Takeda Chemical Industries, Ltd. (Osaka). Doxorubicin (ADM) and 5-fluorouracil (5-FU) were from Kyowa Hakko Kogyo, Co., Ltd. Ftorafur (FT-207, Tegafur, 1-(2-tetrahydrofuryl)-5-fluorouracil) was from Taiho Pharmaceutical Co., Ltd. (Tokyo). Cyclophosphamide (Endoxan, EX) and vincristine sulfate (VCR) were from Shionogi & Co., Ltd. (Osaka). Mitomycin C (MMC) and 1-(4-amino-2-methyl-5-pyrimidinyl)-methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (Nimustine hydrochloride, ACNU) were from Sankyo Co., Ltd. (Tokyo).

THP was dissolved in saline. 6-MP was suspended in 0.5% carboxymethylcellulose - saline solution. Other drugs were dissolved in saline.

Cytotoxicity *In Vitro*

Cytotoxicity of THP, ADM, DM or ACM *in vitro* was studied by examining the inhibition of cell growth and measuring [^{14}C]thymidine or [^{14}C]uridine incorporation using cultured L1210 cells[7].

Antitumor Activity

Male ICR mice, 5 weeks of age, weighing 24~26 g, 10 mice per group, were used for sarcoma-180 or Ehrlich carcinoma experiments. Ascites type cells (5×10^6) were inoculated subcutaneously into the right groin of mice on day 0. Drug solution was injected intraperitoneally or intravenously daily for 7 days from day 1 of the tumor cell inoculation. In the control group, saline solution was given to mice. On day 14, or the indicated day, the mice were killed and the solid tumors were extirpated. Mean tumor weights were evaluated statistically by Student's t-test.

L1210 cells, 1×10^5 in 0.25 ml saline, were inoculated intraperitoneally on day 0 to CDF_1 male mice, weighing 22~25 g, 5 weeks of age. Treatment with several doses was initiated 24 hours later. Combination therapy experiments were performed in 4 treatment schedules (Schedules 1 and 2 for intraperitoneal, and Schedules 3 and 4 for intravenous administration). Schedule 1: THP and combination drugs were injected intraperitoneally once a day from 1 to 10. Schedule 2: THP was injected intraperitoneally on day 1 to 10 but the combination drug was injected on days 1, 5 and 9. Schedule 3: THP and the combination drugs were injected into the tail vein on days 1, 3, 5, 7 and 9. Schedule 4: THP and the combination drugs were given only on day 1. About one half or one third of each optimum doses were setted to the doses of the combination therapy. All drug solution were individually injected without premixing except for the intravenous administration. The injection volume was 0.01 ml/g body weight. Saline (0.25 ml) was given to mice in the control group. Observation was usually terminated on day 60. All mice surviving at that time were recorded as "cured". ILS percents were calculated according to the following equation;

$$ILS\% = (T/C-1)\times100$$

where T is the mean survival time of the treated group and C is that of the control group. Cured mice were excluded from the mean survival time calculation or were included in calculation as having survived only 60 days in the combination therapy, the latter value being expressed in a parenthesis. The survival data were evaluated statistically by Student's t-test. The incidence of cured mice were evaluated statistically by Cai square test.

In order to estimate the relative importance of the combination of these drugs, the combination index (C.I.) was defined as follows;

$$\text{Combination index (C.I.)} = A/B$$

where A is %ILS of combination of THP with other drug and B is the sum of %ILS of single dose of THP and that of other drug.

Results

Effect on Cultured L1210 Cells

Inhibitory effects of THP, ADM, DM and ACM on the growth of cultured L1210 cells and incorporation of [^{14}C]tymidine or [^{14}C]uridine into the cells are shown in Table 1. An IC_{50} value for cell growth, a concentration at which 50% growth inhibition was obtained, was 0.003 μg/ml for THP, 0.016 μg/ml for ADM, 0.012 μg/ml for DM or 0.01 μg/ml for ACM, respectively. The IC_{50} values for [^{14}C]tymidine incorporation were 0.12 μg/ml for THP, 1.4 μg/ml for ADM, 0.42 μg/ml for DM and 0.30 μg/ml for ACM, respectively. For [^{14}C]uridine incorporation, IC_{50} values were 0.06 μg/ml for THP, 0.55 μg/ml for ADM, 0.16 μg/ml for DM and 0.038 μg/ml for ACM, respectively.

Effect on Solid-type Sarcoma-180

THP was active against subcutaneously implanted sarcoma-180 by the intraperitoneal administration daily for 7 days as shown in Table 2. THP produced 58.7%, 68.5%, 83.5% of TIR % at doses of 0.8, 1.6 and 2.4 mg/kg/day, respectively. ADM also produced 44.3%, 57.7%, 72.6% of TIR % at the same doses. There were no complete tumor regressions in the THP- or ADM-treated mice. When THP was given intravenously at doses of 0.2 to 3.2 mg/kg/day daily for 7 days, TIR % of 73.9% and 79.5% were recorded in the groups which received doses of 0.8 and 1.6 mg/kg/day, respectively.

Table 1. Effect of anthracyclines on the growth of L1210 leukemia cells and the incorporation of thymidine and uridine.

Drugs	IC_{50} (μg/ml)			
	Cell growth	Incorporation		
		Thymidine (a)	Uridine (b)	a/b
THP	0.003	0.12	0.06	2.0
ADM	0.016	1.4	0.55	2.5
DM	0.012	0.42	0.16	2.6
ACM	0.01	0.30	0.038	7.9

Table 2. Antitumor effects of THP and ADM on solid-type sarcoma-180.

Treatment route and schedule	Drug	Tumor inhibition ratio (TIR %)						
		Dose (mg/kg/day)						
		0.1	0.2	0.4	0.8	1.6	2.4	3.2
ip, day 1~7	THP	13.3	24.6	45.9	58.7	68.5	83.5	77.5
	ADM	7.6	33.9	39.9	44.3	57.7	72.6	78.1
iv, day 1~7	THP	—	40.9	47.7	73.9	79.5	—	Toxic
	ADM	—	37.3	46.3	62.5	72.0	—	93.8 (Toxic)

Sarcoma-180 (5×10^6 cells) was inoculated subcutaneously into the right groin of ICR mice on day 0. Drug solutions were injected intraperitoneally (ip) or intravenously (iv) daily for 7 days from day 1. On day 14, the mice were killed and solid tumors were extirpated.

Tumor inhibition ratio was calculated on the basis of the mean tumor weights of treated (T) and control (C) group according to the following equation.

$$\text{Tumor inhibition ratio (TIR \%)} = (1 - T/C) \times 100.$$

—: Not tested.

Table 3. Antitumor effects of THP and ADM on solid-type Ehrlich carcinoma.

Treatment route and schedule	Drug	Tumor inhibition ratio (TIR %)						
		Dose (mg/kg/day)						
		0.1	0.2	0.4	0.8	1.6	2.4	3.2
ip, day 1~7	THP	18.3	18.7	28.7	41.3	59.0	61.2	62.4 (Toxic)
	ADM	0.4	28.7	34.5	67.1	77.5	81.1	Toxic
iv, day 1~7	THP	—	22.5	66.5	55.3	70.8	—	Toxic
	ADM	—	18.6	47.1	52.9	74.9	—	89.7 (Toxic)

Ehrlich carcinoma (5×10^6 cells) was inoculated subcutaneously into the right groin of ICR mice on day 0. Drug solutions were injected intraperitoneally (ip) or intravenously (iv) daily for 7 days from day 1. On day 14, the mice were killed and solid tumors were extirpated.

Tumor inhibition ratio (TIR %)=(1−T/C)×100.

—: Not tested.

All mice treated with THP 3.2 mg/kg/day intravenously died due to the toxicity. In the case of ADM, 93.8% TIR % were recorded at the same dose, but many lethal toxic signs were also observed in the mice. There were no significant difference of the mean tumor weight between the received group of THP and that of ADM at the same dose. Consequently, THP showed almost the same activity on solid-type sarcoma-180 as ADM by intraperitoneal or intravenous treatment.

Effect on Solid-type Ehrlich Carcinoma

The activity of THP was also compared with that of ADM in mice inoculated subcutaneously with Ehrlich carcinoma. As shown in Table 3, THP produced 41.3%, 59.0% and 61.2% of TIR % at doses of 0.8, 1.6 and 2.4 mg/kg/day, respectively, by intraperitoneal administration daily for 7 days. In the case of ADM, 67.1%, 77.5%, 81.1% of TIR % were observed at the same dose levels. When THP was administered intravenously, daily for 7 days, 55.3% and 70.8% TIR % values were obtained at doses of 0.8 and 1.6 mg/kg/day, respectively. In the case of ADM, 52.9% and 74.9% were observed at the same dose levels. At a dose of 3.2 mg/kg/day of THP, there were mice killed due to drug toxicity within the 14 day-observation period. ADM, at 3.2 mg/kg/day, achieved a TIR of 89.7%, however, many lethal signs were also observed in these treated mice. Thus, antitumor activities of THP and ADM were almost the same against Ehrlich carcinoma implanted subcutaneously in ICR mice when treated intraperitoneally or intravenously.

Dose Schedule Dependency of the Antitumor Effect on Sarcoma-180 and Ehrlich Carcinoma

Dose schedule dependency of THP or ADM was studied with their antitumor effects on sarcoma-180 or Ehrlich carcinoma as shown in Table 4. All therapies were compared to each other at the same total cumulative dose (7.0 mg/kg). When THP was given once daily for 5 days, from day 1 (Schedule 1-A), day 7 (Schedule 1-B), or day 14 (Schedule 1-C), it produced similar TIR % against sarcoma-180 in Schedules 1-A and 1-C group (51.6% and 48.9%). But we observed higher activity against Ehrlich carcinoma using Schedule 1-B (56%) than Schedule 1-A (46.4%). On the other hand, ADM showed higher activity against the two solid type tumors using Schedule 1-A than the other two schedules. When THP or ADM was injected twice, namely day 2 and day 4 (Schedule 2-A), day 7 and day 11 (Schedule 2-B) or day 14 and day 18 (Schedule 2-C), only the mice treated with THP or ADM on Schedule 2-A showed more than a 50% TIR. The other two dose schedule produced only 4.7~35.8% TIR with both THP and ADM. In case of single dose, the TIR % values of the two drugs

Table 4. The influence of the treatment schedule (intravenous administration) on the antitumor effect of THP against solid-type sarcoma-180 and Ehrlich carcinoma.

	Administration schedule (days)																		Tumor inhibition ratio (TIR %)			
																			S-180 solid tumor		Ehrlich solid tumor	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	THP	ADM	THP	ADM
1-A	↑	↑	↑	↑	↑	·	·	·	·	·	·	·	·	·	·	·	·	·	51.6	58.0	46.4	48.0
1-B	·	·	·	·	·	·	↑	↑	↑	↑	↑	·	·	·	·	·	·	·	26.7	39.5	56.0	33.1
1-C	·	·	·	·	·	·	·	·	·	·	·	·	·	↑	↑	↑	↑	↑	48.9	22.8	24.5	17.4
2-A	·	↑	·	↑	·	·	·	·	·	·	·	·	·	·	·	·	·	·	60.4	55.5	53.7	54.2
2-B	·	·	·	·	·	·	↑	·	·	·	↑	·	·	·	·	·	·	·	14.8	21.2	4.7	35.8
2-C	·	·	·	·	·	·	·	·	·	·	·	·	·	↑	·	·	·	↑	21.3	33.1	12.5	23.8
3-A	↑	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	62.1	55.7	59.9	67.8
3-A′	·	·	·	·	↑	·	·	·	·	·	·	·	·	·	·	·	·	·	25.3	27.1	32.8	25.3
3-B	·	·	·	·	·	·	↑	·	·	·	·	·	·	·	·	·	·	·	30.3	35.9	67.7	14.7
3-C	·	·	·	·	·	·	·	·	·	·	·	·	·	↑	·	·	·	·	11.7	15.8	10.7	11.3

Inoculation: 5×10^6 cells/mouse/0.2 ml (day 0).
Evaluation: Day 14 (group A), day 21 (group B), day 28 (group C).
Therapy: 7 mg/kg (total), intravenously.
↑: Treatment day.

Table 5. Antitumor effect of THP on L1210 leukemia (ip-ip).

Dose (mg/kg/day)	THP		ADM	
	ILS %	Cured mice	ILS %	Cured mice
0.078	12		22	
0.156	29		34	
0.31	40		54	
0.625	107		68	
1.25	194 (297)	5/18	178 (192)	1/6
2.5	179 (375*)	7/18	114	
3.75	69		54	
5.0	25		35	

L1210 cells (1×10^5) were inoculated intraperitoneally to CDF_1 mice on day 0. THP and ADM was injected intraperitoneally once a day from day 1 to 10.

( ); ILS % in which included cured mice as 60-day survivors.

* Significantly different from ADM ($p<0.05$).

Table 6. Antitumor effect of the single intravenous injection of THP on L1210 leukemia.

Dose (mg/kg)	Increase of life span (ILS %)					
	Day 1		Day 3		Day 5	
	THP	ADM	THP	ADM	THP	ADM
1.56	11	—	8	—	—	—
3.12	21	—	23	—	17	—
6.25	66	25	63	19	27	16
12.5	170*	58	185*	40	52	25
25.0	—	—	235*	38	50	—

L1210 cells (1×10^5) were inoculated intraperitoneally to CDF_1 mice on day 0. THP or ADM was injected intravenously on day 1 or day 3 or day 5.

—: Not tested.

* Significantly different from ADM ($p<0.05$).

were highest in Schedule 3-A group (treated on day 1) and decreaesd with delay of the treatment, although THP showed 67.7% TIR by treatment on day 7 against Ehrlich carcinoma.

Effect on Murine Leukemia L1210

Mean survival times (MST) of mice bearing L1210 were prolonged by 107, 194 (5/18, 297) and 179% (7/18, 375%) at daily doses of 0.625, 1.25 and 2.5 mg/kg/day, respectively, when treated intraperitoneally with THP for 10 consecutive days from day 1, as shown in Table 5. A critical prolongation of the MST for efficacy in this system (25% ILS) was observed at a daily dose of 0.156 mg/kg/day. ADM was also active against L1210 at doses between 0.156 mg/kg/day and 2.5 mg/kg/day and its maximum effectiveness (178, 1/6, 192) was obtained at a daily dose of 1.25 mg/kg/day.

Results of single bolus intravenous administration of THP or ADM are shown in Table 6. Treatment on day 1 gave a maximum ILS % of 170 at a dose of 12.5 mg/kg of THP. But at the same dose of ADM 58% ILS was given. When the treatment was carried out 3 days after implantation, the mice receiving THP at doses of 6.25, 12.5 and 25 mg/kg, showed 63%, 185% and 235% ILS, respectively. ADM produced 19, 40 and 38% ILS at these doses. Therefore ILS caused by THP were greater than those found in ADM at every dose given by intravenous administration.

Dose schedule dependency was examined by twice intraperitoneal or intravenous treatment

Table 7. Antitumor effect of twice intravenous or intraperitoneal injections of THP and ADM on L1210 leukemia.

Treatment schedule	Treatment route, ip				Treatment route, iv			
	THP		ADM		THP		ADM	
	ILS %	Cures	ILS %	Cures	ILS %	Cures	ILS %	Cures
Day 1+day 1	(390)	(6/6)	120	(3/6)	117	(4/6)	92	(0/6)
Day 1+day 2	292	(5/6)	118	(1/6)	183	(4/6)	113	(0/6)
Day 1+day 3	222	(3/6)	173	(3/6)	146	(2/6)	72	(0/6)
Day 1+day 4	132	(4/6)	208	(1/6)	85	(0/6)	64	(0/6)
Day 1+day 5	221	(0/6)	163	(2/6)	143*	(0/6)	24	(0/6)
Day 1+day 6	273*	(4/6)	86	(0/6)	158*	(0/6)	60	(0/6)
Day 1+day 7	243	(3/6)	114	(2/6)	161*	(1/6)	54	(0/6)

Tumor: L1210, 1×10^5 cells/mouse, ip on day 0.
Treatment: THP or ADM, 6.25 mg/kg/shot (total dose 12.5 mg/kg).
Cures: 40-day survivors.
* Significantly different from ADM ($p<0.05$).

(Table 7). The intraperitoneal injection of THP (6.25 mg/kg×2) produced 200~390 ILS % in all combinations of treatment days except for the combination of day 1 and day 4. On the other hand, ADM showed similar efficacy as THP in the treatment at the early stage but treatments on day 5, day 6 or day 7 in combination with day 1 were less effective than THP. In case of intravenous treatment, THP produced 4/6, 4/6 or 2/6 of cured mice when it was injected on day 1, day 2 or day 3, respectively, in addition to day 1. But there was no cured mice in the groups treated with ADM using the same doses and schedules.

Effect of THP in Combination with Other Antitumor Agent on Murine L1210 Leukemia

Combination effect of THP with other antitumor agents on L1210 were examined by intraperitoneal treatment on Schedules 1 and 2 and intravenous treatment on Schedules 3 and 4.

The combination of THP with Ara-C, Cyclo-C, 6-MP or EX showed a high effectiveness when the two drugs were administered intraperitoneally once a day for 10 days (Schedule 1) as shown in Table 8. THP (0.625 mg/kg/day) given in combination with Ara-C (5 mg/kg/day) produced 361% ILS (3/6 of cured mice, 498%, C.I.=3.5), which was greater than that of the individual ILS % of THP (97%) or Ara-C (47%). Similarly, the combined treatment of THP with Cyclo-C (25 mg/kg/day) gave 219% ILS (2/6 of cured mice, 358%, C.I.=2.3) and its value was greater than that of individual each agent at that doses. Combination therapy of THP with EX (25 mg/kg/day) produced 235% ILS (4/6, 490%, C.I.=2.4) and it was also superior to each value obtained by single use of THP (97%) or EX (104%). Of these three drugs, many cured mice appeared in combination with THP at its dose level of 1.25 mg/kg/day. THP also produced highly effectiveness by the combined dose of 0.625 mg/kg/day of THP and 10 or 20 mg/kg/day of 6-MP (C.I.=2.4~3.4). Combination with MTX (0.5 mg/kg/day), MMC (0.5 mg/kg/day), VCR (0.25 mg/kg/day) or ACNU (1.25 mg/kg/day) produced 152% (1/6, 233%, C.I.=1.4), 164%, (C.I.=1.3), 148% (2/6, 310%, C.I.=2.1) or 221% (1/6, 301%, C.I.=2.4), respectively, in the combined dose of 0.625 mg/kg/day of THP. In case of 5-FU or FT207, however, the combination effect was not obtained.

Treatment on Schedule 2 showed a high effectiveness in combination with Ara-C, Cyclo-C,

Table 8. Antitumor effect of THP in combination with 10 various antitumor agents (intraperitoneal administration for 10 days, Schedule 1).

Drug	Dose (mg/kg/day)	THP (mg/kg/day)				
		0	0.625		1.25	
		ILS %	ILS %	C.I.	ILS %	C.I.
THP alone	—	—	97		169 (4/18, 321)	
Ara-C	5	47	361 (3/6, 498)	3.5	160 (2/6, 318)	0.9
	10	211 (1/6, 282)	212 (2/6, 353)	0.9	249 (4/6, 506)	0.8
Cyclo-C	25	60	219 (2/6, 358)	2.3	219 (5/6, 567)	1.5
	50	105	178 (3/6, 408)	2.0	637 (6/6, 637)	1.5
6-MP	10	10	195 (1/6, 262)	2.4	191 (2/6, 325)	1.0
	20	33	296 (3/6, 444)	3.4	241 (2/6, 340)	1.0
MTX	0.5	67	152 (1/6, 233)	1.4	191 (1/6, 265)	0.7
	1.0	104	155	0.8	184	0.4
5-FU	12.5	39	98 (1/6, 188)	1.4	141	0.4
	25	90	135	0.7	108	0.3
FT207	100	14	137	1.2	147	0.4
	200	63	41	0.3	22	0.1
EX	12.5	35	155	1.2	230 (2/6, 394)	1.1
	25	104	235 (4/6, 490)	2.4	181 (5/6, 559)	1.3
MMC	0.25	21	77	0.7	240	0.7
	0.5	30	164	1.3	240 (1/6, 321)	0.9
VCR	0.25	51	148 (2/6, 310)	2.1	187 (1/6, 261)	0.7
	0.5	76	188	1.1	120	0.3
ACNU	1.25	28	221 (1/6, 301)	2.4	266 (2/6, 384)	1.1
	2.5	80	269 (2/6, 386)	2.2	193 (1/6, 264)	0.7

L1210 cells (1×10^5) were inoculated intraperitoneally to CDF_1 mice on day 0.
THP and the combination drug were injected intraperitoneally once a day from day 1 to 10.
( ): Cured mice/treated mice, ILS % which included cured mice as 60-day survivors.
C.I.: Combination index $=\frac{(\%\text{ ILS of combination of THP with other drug})}{(\%\text{ ILS of THP})+(\%\text{ ILS of combined drug})}$

EX or MMC, where THP was administered at a dose of 0.625 mg/kg/day intraperitoneally once a day from day 1 to 10 and each combined drug was given intraperitoneally on days 1, 5 and 9 (Table 9). The combination of THP with Ara-C produced 273% ILS (4/6 of cured mice, 514%, C.I.=2.6) and its ILS % was greater than that of individual ILS% of THP (157%) or Ara-C (43%). Similarly, the combination of THP with Cyclo-C, MMC, or EX also produced 292% ILS (3/6 of cured mice, 463, C.I.=2.3), 260% (3/6, 440, C.I.=1.8) or 624% (6/6, C.I.=2.2), respectively, which was greater than those produced by the treatment with each single agent (45% by Cyclo-C, 92% by MMC, 133% by EX).

THP showed a high effectiveness on L1210 even by the intravenous treatment in combination with Ara-C or EX on Schedules 3 and 4 (Tables 10 and 11). The combination of THP with Ara-C produced 343% ILS (3/6 of cured mice, 475, C.I.=4.4), where Ara-C alone (20 mg/kg/day) or THP alone (1.25 mg/kg/day) showed 53% or 56% ILS, respectively. Especially, 2/6, 3/6 and 2/6 of cured mice were observed in the Ara-C combination groups. As same as in the intraperitoneal administration combined treatment with EX also produced a high effectiveness. When 2.5 mg/kg/day of THP and 50 mg/kg/day of EX were administered, all treated mice were cured (Table 10). On the other hand, the combination with Cyclo-C was not so effective as in the intraperitoneal administration of the same drug combination. VCR and 5-FU did not show greater effectiveness than THP alone.

Table 9. Antitumor effect of THP in combination with various antitumor agents (intraperitoneal intermittent administration, Schedule 2).

Drug	Dose (mg/kg/day)	THP (mg/kg/day)		
		0	0.625	
		ILS %	ILS %	C.I.
THP alone	—	—	157	
Ara-C	10	31	349 (3/6, 492)	2.6
	20	43	273 (4/6, 514)	2.6
Cyclo-C	50	—	145 (5/6, 553)	—
	100	45	292 (3/6, 463)	2.3
MTX	1	44	171 (1/6, 246)	1.2
	2	54	178 (1/6, 252)	1.2
5-FU	25	—	124 (209, 1/6)	—
	50	72	158	0.7
FT207	200	—	236 (1/6, 303)	—
	400	78	62	0.3
EX	25	51	147	0.7
	50	133	624 (6/6)	2.2
MMC	1	48	287 (2/6, 398)	1.9
	2	92	260 (3/6, 440)	1.8
VCR	0.5	—	174 (0/6)	—
	1.0	38	134 (0/6)	0.7
CHR	0.5	—	71	0.4
	1.0	51	116 (1/6, 200)	1.0

L1210 cells (1×10^5) were inoculated intraperitoneally to CDF_1 mice on day 0. THP was injected intraperitoneally once a day from day 1 to 10 and the combination drug was injected on days 1, 5 and 9 (Schedule 2).

( ): Cured mice/treated mice, ILS % which included cured mice as 60-day survivors.

C.I.: Combination index $=\frac{(\%\text{ ILS of combination of THP with other drug})}{(\%\text{ ILS of THP})+(\%\text{ ILS of combined drug})}$

Table 10. Antitumor effect of THP in combination with Ara-C, Cyclo-C, EX, VCR or 5-FU (intermittent intravenous administration on days 1, 3, 5, 7 and 9, Schedule 3).

Drug	Dose (mg/kg/day)	THP (mg/kg/day)				
		0	1.25		2.5	
		ILS %	ILS %	C.I.	ILS %	C.I.
THP alone	—	—	56		138	
Ara-C	10	33	218 (2/6, 347)	3.9	259 (2/6, 375)	2.2
	20	53	343 (3/6, 475)	4.4	222	1.2
Cyclo-C	50	35	96	1.1	135	0.8
	100	49	129	1.2	135	0.7
EX	25	79	131	1.0	185	0.9
	50	135	233 (1/6, 302)	1.6	650 (6/6)	2.4
VCR	0.3	2	43	0.7	90	0.6
	0.6	7	47	0.8	96	0.7
5-FU	25	51	69	0.6	86	0.5
	50	59	33	0.3	41	0.2

L1210 cells (1×10^5) were inoculated intraperitoneally to CDF_1 mice on day 0.

THP and the combination drug were injected intravenously on days 1, 3, 5, 7 and 9 (Schedule 3).

( ): Cured mice/treated mice, ILS % which included cured mice as 60-day survivors.

C.I.: Combination index $=\frac{(\%\text{ ILS of combination of THP with other drug})}{(\%\text{ ILS of THP})+(\%\text{ ILS of combined drug})}$

Table 11. Antitumor effect of THP in combination with Ara-C, Cyclo-C, EX, VCR or 5-FU (single intravenous administration, Schedule 4).

Drug	Dose (mg/kg/day)	THP (mg/kg)				
		0	6.25		12.5	
		ILS %	ILS %	C.I.	ILS %	C.I.
THP alone	—	—	88		227 (1/12, 239)	
Ara-C	50	12	198	2.0	144 (3/6, 382)	1.5
	100	14	158	1.5	248 (5/6, 558)	2.2
Cyclo-C	150	19	104	1.0	308 (1/6, 365)	1.4
	300	21	88	1.4	298	0.8
EX	62.5	57	439 (5/6, 602)	4.2	378 (4/6, 583)	2.0
	125	112	322 (4/6, 531)	2.7	635 (6/6)	1.8
VCR	0.63	6	94	1.0	214 (1/6, 277)	1.1
	1.25	4	85	0.9	188 (2/6, 323)	1.3
5-FU	62.5	22	82	0.7	239	0.9
	125	39	100	0.8	108	0.4

L1210 cells (1×10^5) cells were inoculated intraperitoneally to CDF_1 mice on day 0.
THP and the combination drugs were injected intravenously on day 1 (Schedule 4).
( ): Cured mice/treated mice, ILS % which included cured mice as 60-day survivors.
C.I.: Combination index $=\frac{(\%\text{ ILS of combination of THP with other drug})}{(\%\text{ ILS of THP})+(\%\text{ ILS of combined drug})}$

Table 11 showed the results of the same drug combination by single intravenous administration (Schedule 4). The single dose of THP on day 1 produced 88% ILS at dose of 6.25 mg/kg. The combination of THP with Ara-C gave 198% ILS (C.I.=2.0) at a dose of 50 mg/kg/day. The combination of THP with EX also gave 439% ILS (5/6 of cured mice, 602, C.I.=4.2) and 322% (4/6 of cured mice, 531, C.I.=2.7) at a dose of 6.25 mg/kg of THP. But the combination of THP with 5-FU or VCR did not show greater effectiveness than THP alone.

Discussion

THP showed a higher inhibitory effect on the cell growth of cultured L1210 cells *in vitro* than DM, ADM and ACM. The nucleic acid synthesis, *i.e.*, incorporation of [^{14}C]tymidine or [^{14}C]uridine into the cultured cells was also markedly inhibited by this drug. A ratio of IC_{50} for the former to that for the latter was 2.0. Namely THP inhibits DNA and RNA syntheses at the almost same drug concentration. Therefore it would be classified like ADM and DM as a Class I anthracycline according to CROOKE *et al.*[8].

ADM has been known to be highly active on experimental solid-type tumors[9~11]. THP, in the comparative studies with ADM, showed almost the same activity on solid-type sarcoma-180 and Ehrlich carcinoma. In addition, THP gave a superior growth inhibitory effect compared to ADM when given by intravenous treatment against sarcoma-180 in the later stage of tumor growth. This suggests the possible efficacy of THP on some tumors in their advanced stages of growth.

High antitumor activity of THP on murine leukemia has been reported[1,2]. In the present study, the high efficacy of THP on L1210 was confirmed. Especially, cured mice and long-term survivors, were observed with a high frequency even in the intravenous treatment at the early stage of tumor growth (day 1 and day 1 or day 1 and day 2) and with a booster shot at the late stage (day 1 and day 7). But intravenous treatment with ADM gave no mice cures. THP exhibited high effectiveness with cocurrent administration of Ara-C or EX. Especially, in case of single intravenous administration on day 1, combination of THP with EX produced 80% cured mice (19/24). Combination of THP with Ara-C, by intraperitoneal administration for 10 days (day 1 to 10), also produced 46% cured mice (11/24)

and intravenous intermittent administration (on days 1, 3, 5, 7 and 9) also produced 29% cured mice (7/24).

Combination with Cyclo-C, by intraperitoneal administration for 10 days or days 1, 5 and 9 produced high therapeutic effectiveness; particularly, the 10 day-administration produced 67% cured mice (16/24). However the 5 times intermittent intravenous administration showed the same activity as THP alone. Pharmacodynamics of Cyclo-C probably affects the combination therapy.

Clinically, 6-MP has been used widely in combination therapy, namely, DCMP therapy (DM, 6-MP, Ara-C and prednisolone) for treatment of acute lymphatic leukemia and/or acute myeloid leukemia. In our studies, 33% mice (8/24) were cured in the combination of THP with 6-MP by intraperitoneal 10 day-administration. Therefore combination of the three drugs, THP, 6-MP and Ara-C, has hopeful prospect and is under testing. The combination of THP with MMC showed high effectiveness when it was administerad on days 1, 5 and 9 intraperitoneally. The combination with 5-FU and FT207 showed the same effectiveness as THP alone in our experiments.

AVERY and ROBERTS[12] had shown that pronounced therapeutic effectiveness of the combination of ADM with EX was attributed to a true potentiation of the independent oncolytic action of each agents.

HARTMAN *et al.*[13] reported an effect of EX pretreatment on the short term disposition and biliary excretion of ADM metabolites in rat. There was a significant decrease in a plasma ADM clearance and 83% increase in bile flow. It is of interest whether the distribution and metabolism of THP may be affected by simultaneous injection of EX.

ROBERTS *et al.*[14] had reported the therapeutic effectiveness in the combination therapy of ADM with Ara-C. They suggested that varied dosages of oncolytic drugs could be used to produce equivalent therapeutic response and that the order of administration may influence over all effectiveness of sequential multiple-drug therapy.

The mechanism of action of THP, *i.e.*, inhibition of the synthesis of DNA is similar to ADM[15]. However, it has been reported that no major part of THP is metabolized to ADM but intact THP directly inhibits DNA synthesis[4,15]. It has been reported that the metabolism and disposition of THP is different from that of ADM *in vivo*[4]. These suggest different and hopefully better effects of THP on certain tumors from that of especially ADM in combination therapy.

Our present results indicate the therapeutic effectiveness of THP in combination with EX, Ara-C, or Cyclo-C and provide some hopeful prospects for clinical combination therapy of the new drug.

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