STUDIES ON ANTI-TUMOR AGENTS. III

Anti-tumor Activity of a Triazylstilbene Derivative (TC-17)

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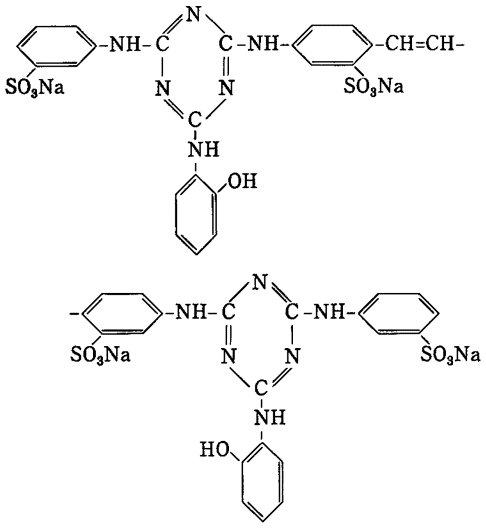
(Received December 5, 1968)

Introduction

Since DE BRUYN *et al.*[1] reported the affinity of fluorescent dyes, diaminoacridines, for nucleoproteins in mammalian cells in 1953, a number of biological and biochemical studies[2~14] on cancer of fluorescent dyes of various chemical structures have been performed and anti-tumor activities of these fluorescent dyes have been reported by KORGAONKAR & SUKHATANKAR and HATTORI[16].

During the course of investigations of the anti-tumor activity of various types of fluorescent dyes in our laboratory, it has been found that one of triazylstilbene derivatives showed a marked tumor-inhibiting activity when the treatment was given prior to tumor cell inoculation and was extremely low toxic.

The compound, TC-17 has a weak fluorescence and its chemical structure is as follows ;



TC-17

4,4′-bis(2-0-oxyanilino-4-*m*-sulfoanilino-1,3,5-triazyl-6)stilbene disulfonate-2,2′

The present paper concerns the anti-tumor activity of TC-17 in comparison with that of mitomycin C and cyclophosphoramide.

Materials and Methods

Compound Tested :

TC-17.....Light yellow crystal. Water solution shows a yellow fluorescence with activation maximum at the wave length of 370 mμ and fluorescence maximum at the wave length of 410 mμ.

Mitomycin C....."Mitomycin-Kyowa S-" (designated as MMC)

Cyclophosphamide....."Endoxan -Shionogi-" (designated as CPM)

The compounds were dissolved in physiological saline solution and the solutions were freshly prepared before use.

Anti-tumor Test :

Ehrlich carcinoma, sarcoma 180 and MH 134 hepatoma in mice, and Yoshida sarcoma in rats were chosen for the present study. Ehrlich carcinoma or sarcoma 180 and MH 134 hepatoma were transplanted into ICR-JCL (male, 6 weeks old) or C3H (male, 6 weeks old) mice respectively, and Yoshida sarcoma was transplanted into Donryu rats (male, 5 weeks old). In the experiments, five to seven mice or rats per each group of tests were used. Solid tumors were produced by subcutaneous injection of fresh ascitic fluid containing tumor cells (2×10^6/mouse, 10^7/rat) into inguinal region of experimental animals, and ascites tumors were produced by intraperitoneal injection of tumor cells (2×10^6) into mice and rats.

Compounds were administered for 7 successive days before or after tumor inoculation except where otherwise noted. The first injection of compounds was started 24 hours after tumor inoculation in the case of post-treatment, and the tumor cells were implanted 24 hours after the last injection of compounds in the case of pretreatment and the

tumor bearing animals were left without any further treatment.

In solid tumors, all mice or rats were killed on the 8th day after tumor inoculation and weights of the tumors were measured, and the effect of compound was expressed as the inhibition ratio (IR) which was calculated from the following equation.

$$IR = (1 - Tw/Cw) \times 100$$

Cw : the mean control-tumor weight

Tw : the mean treated-tumor weight

In ascites tumors, the effect of compound was expressed as the increase in life-span (ILS) of experimental animals which was calculated from the following equation.

$$ILS = (Ts/Cs - 1) \times 100$$

Cs : the mean survival days of control group

Ts : the mean survival days of treated group

Toxicity :

The acute toxicity was determined by various routes of administration in male albino mice weighing 15~20 g and male albino rats weighing about 200 g. Each group consisted of ten mice or rats. The LD_{50} observed during ten days was calculated by the method of LITCHFIELD and WILCOXON[17].

Blood Test :

Rabbits were used in this experiment. White blood cell count, red blood cell count and platelet count were determined by routine procedure on peripheral blood taken from the ear vein of animals, and the average values of each group consist of two or five rabbits were calculated.

Results

Anti-tumor Activity :

Table 1 shows the effects of TC-17 with post-treatment on various solid tumors. In a dose of 50~75 mg/kg, TC-17 showed a moderate inhibition from about 30 to 60% in IR on various tumors when the treatment was started parenterally 24 hours after tumor inoculation. When the treatment was initiated on the 7th day after the implantation of Ehrlich carcinoma or sarcoma 180, the antitumor

Table 1. Effect of TC-17 with post-treatment on solid tumors

Tumor	Compd.	Dose (mg/kg/day)	Route	Day the treatment started	Mortality	Av. body wt. change (g) T/C*	Av. tumor wt. (g) Tw/Cw**	IR*** (%)
Ehrlich carcinoma	TC-17	50×7	ip	1st	0/7	+ 9.1/+10.0	2.34/3.53	33.7
		75 〃		〃	0/7	+ 2.7/+10.0	1.59/3.53	55.0
		50 〃	iv	〃	0/7	+ 6.6/+ 9.9	3.05/4.13	26.1
		75 〃		〃	0/7	+ 4.9/+ 9.9	2.02/4.13	51.2
		50 〃	sc	〃	0/6	+ 7.6/+10.2	2.11/3.02	30.1
		75 〃		〃	0/6	+ 2.3/+10.2	1.80/3.02	40.4
		500 〃	po	〃	0/7	+ 0.8/+ 4.6	3.35/3.46	3.2
	TC-17	100×7	ip	7th	0/7	+ 9.3/+15.0	4.71/7.36	36.0
	MMC	3 〃		〃	0/7	+ 6.6/+15.0	3.75/7.36	49.0
	CPM	80 〃		〃	0/7	+ 2.4/+15.0	3.17/7.36	56.7
Sarcoma 180	TC-17	50×7	ip	1st	0/6	+ 5.8/+ 8.7	1.53/2.67	50.2
		75 〃		〃	0/6	+ 4.0/+ 8.7	0.88/2.67	67.0
		50 〃	iv	〃	0/7	+ 3.6/+ 6.2	1.97/3.17	37.9
		75 〃		〃	0/7	+ 0.1/+ 6.2	1.37/3.17	52.1
		50 〃	sc	〃	0/6	+ 8.5/+ 8.0	1.83/2.46	25.6
		75 〃		〃	0/6	+ 2.3/+ 8.0	1.30/2.46	47.2
		500 〃	po	〃	0/7	+ 4.3/+ 2.7	2.59/3.02	14.2
	TC-17	100×7	ip	7th	0/6	+ 5.4/+ 8.6	2.68/4.01	34.6
	MMC	3 〃		〃	0/6	+ 6.0/+ 8.6	2.30/4.01	42.6
	CPM	80 〃		〃	0/6	+ 7.5/+ 8.6	2.13/4.01	46.7
MH 134	TC-17	25×7	ip	1st	0/7	+ 1.7/+ 2.5	1.30/1.80	27.8
		50 〃		〃	0/7	+ 1.1/+ 2.5	0.79/1.80	56.1
Yoshida sarcoma	TC-17	50×7	ip	1st	0/8	+12.3/+16.1	0.93/1.24	25.0
		100 〃		〃	0/8	+ 1.9/+16.1	0.79/1.24	36.2

* Treated group/Control group

** The mean treated-tumor weight/The mean control-tumor weight

*** Inhibition ratio : $(1 - Tw/Cw) \times 100$

Table 2. Effect of TC-17 with pretreatment on solid tumor

Tumor	Compd.	Dose (mg/kg/day)	Route	Day the treatment started	Mortality	Av. body wt. change (g) T/C*	Av. tumor wt. (g) Tw/Cw**	IR*** (%)
Ehrlich carcinoma	TC-17	50×7	ip	7th day before	0/6	+13.7/+18.2	0.52/3.33	84.5
		75 〃		〃	0/6	+14.0/+18.2	0.31/3.33	90.4
		50 〃	iv	〃	0/6	+ 7.4/+18.6	0.66/2.56	74.2
		75 〃		〃	1/6	− 2.2/+18.6	0.21/2.56	91.7
		50 〃	sc	〃	0/6	+19.8/+18.9	1.55/2.89	46.4
		75 〃		〃	0/6	+ 9.7/+18.9	0.98/2.89	66.2
	MMC	3 〃	ip	〃	1/7	+ 5.5/+13.5	1.09/2.74	60.0
	CPM	80 〃		〃	3/7	+ 6.8/+13.5	1.29/2.74	52.6
	TC-17	50 〃	ip	14th day before	0/7	+13.7/+18.6	0.80/1.40	43.2
		75 〃		〃	0/7	+ 3.3/+16.9	0.78/4.65	83.6
	MMC	3 〃	ip	〃	6/7	—	—	—
	CPM	80 〃		〃	3/7	+ 0.9/+15.8	1.00/1.84	45.7
	TC-17	50 〃	ip	21st day before	0/7	+11.9/+23.8	2.05/3.31	38.1
		75 〃		〃	2/7	+ 0.3/+23.8	0.73/3.31	78.0
	CPM	80 〃	ip	〃	5/7	—	—	—
Sarcoma 180	TC-17	50×7	ip	7th day before	0/6	+10.6/+16.0	0.57/1.38	58.6
		75 〃		〃	0/6	+ 5.0/+16.0	0.26/1.38	81.2
		50 〃	iv	〃	0/6	+ 5.3/+16.3	0.72/1.99	64.0
		75 〃		〃	0/6	− 2.9/+16.3	0.14/1.99	93.0
		50 〃	sc	〃	0/6	+ 6.4/+17.3	0.46/1.63	72.0
		75 〃		〃	0/6	+ 3.2/+17.3	0.22/1.63	86.7
	MMC	3 〃	ip	〃	2/7	+ 7.3/+14.6	1.53/2.87	46.7
	CPM	80 〃		〃	3/7	+ 6.7/+14.6	1.51/2.87	47.4
	TC-17	50 〃	ip	14th day before	0/7	+ 6.0/+16.6	0.47/1.23	61.8
		75 〃		〃	0/7	+ 0.5/+10.1	0.39/2.28	82.9
	MMC	3 〃	ip	〃	1/7	+ 8.7/+14.2	0.76/1.28	40.6
	CPM	80 〃		〃	2/7	+ 7.8/+14.2	0.87/1.28	32.3
	TC-17	50 〃	ip	21st day before	0/7	+10.1/+15.3	1.87/2.53	26.1
		75 〃		〃	1/7	+ 1.1/+15.3	0.93/2.53	63.2
	CPM	80 〃	ip	〃	1/7	+11.1/+15.3	1.87/2.53	26.1
MH 134	TC-17	25×7	ip	7th day before	0/7	+ 1.5/+ 2.7	0.82/1.44	43.0
		50 〃		〃	0/7	+ 0.1/+ 2.7	0.61/1.44	57.6
Yoshida sarcoma	TC-17	50×7	ip	7th day before	0/8	+ 0.5/+56.0	0.79/1.78	55.5
		100 〃		〃	0/8	− 6.0/+56.0	0.49/1.78	72.6

* Treated group/Control group

** The mean treated-tumor weight/The mean control-tumor weight

*** Inhibition ratio : $(1-Tw/Cw)\times 100$

activity of TC-17 (100 mg/kg) was less effective than those of mitomycin C (3 mg/kg) and cyclophosphamide (80 mg/kg) under the same condition. TC-12 was equally active when it was given intraperitoneally, intravenously or subcutaneously, but it was found that there was little or no effect with oral administration.

Table 2 shows the effect of TC-17 with pretreatment on various solid tumors. When tumor cell inoculation was performed 24 hours after treatments of drugs, TC-17 (50~75 mg/kg) without significant toxicity showed tumor-inhibiting activity considerably superior to that of mitomycin C (3 mg/kg), cyclophosphamide (80 mg/kg) on Ehrlich carcinoma or sarcoma 180 in mice. Furthermore, even if tumor cell inoculation was performed one or two weeks after the last injection of the drug, the tumor-inhibiting activity of TC-17 (75 mg/kg) continued the IR of about 60% or more. The prolonged duration of effect was one of the most remarkable characteristics. Activities of TC-17 with pretreatment on the growth of MH 134 hepatoma or Yoshida sarcoma were less effective than that of Ehrlich carcinoma or sarcoma 180.

Relationship between tumor-inhibiting activity and numbers of injection of TC-17 with pretreatment

Table 3. Relationship between effects and numbers of injection of TC-17 on tumor growth in mice (pretreatment)

Tumor	Dose (mg/kg/day)	Numbers of injection (ip)	Mortality	Av. body wt. change (g) *T/C**	Av. tumor wt. (g) *Tw/Cw***	IR**** (%)
Ehrlich carcinoma	75	1	0/6	+17.3/+18.4	1.88/2.34	19.6
		2	0/6	+12.8/+18.4	1.31/2.34	39.5
		3	0/6	+19.9/+18.4	1.19/2.34	49.2
		4	0/6	+13.1/+18.4	0.69/2.34	70.5
		7	0/6	+ 4.2/+18.4	0.42/2.34	82.1
Sarcoma 180	75	1	0/6	+13.8/+13.9	1.59/1.65	1.8
		2	0/6	+10.9/+13.9	1.25/1.65	24.2
		3	0/6	+ 7.2/+13.9	0.60/1.65	63.7
		4	0/6	+ 5.0/+13.9	0.55/1.65	66.6
		7	0/6	+ 0.3/+13.9	0.26/1.65	84.2

* Treated group/Control group
** The mean treated-tumor weight/The mean control-tumor weight
*** Inhibition ratio $(1-Tw/Cw)\times 100$

Table 4. Effect of TC-17 following successive intraperitoneal injections on solid tumors

Tumor	Dose (mg/kg/day)	Day the treatment started	Mortality	Av. body. wt. change (g) *T/C**	Av. tumor wt. (g) *Tw/Cw***	IR**** (%)
Ehrlich carcinoma	50×21	1st	0/7	+16.0/+21.6	12.36/18.44	32.9
Sarcoma 180	50×21	1st	0/7	+15.6/+18.5	7.90/13.75	43.1

* Treated group/Control group
** The mean treated-tumor weight/The mean control-tumor weight
*** Inhibition ratio : $(1-Tw/Cw)\times 100$

Table 5. Combination of TC-17 and other anti-tumor agents on subcutaneously implanted tumor in mice

Tumor	Dose (mg/kg/day) TC-17 (ip)	MMC (sc)	CPM (sc)	No. of injections	Mortality	Av. body wt. change (g) *T/C*	Av. tumor wt. (g) *Tw/Cw*	IR (%)
Ehrlich carcinoma	10			7	0/7	+ 8.0/+8.1	2.99/2.88	−3.8
	25			7	0/7	+11.6/+8.1	2.84/2.88	1.4
		0.5		7	0/7	+10.7/+8.1	2.93/2.88	−3.6
			10	7	0/7	+10.1/+8.1	2.44/2.88	15.3
	10	0.5		7	0/7	+ 7.3/+8.1	1.92/2.88	33.4*
	25	0.5		7	0/7	+ 7.0/+8.1	1.80/2.88	37.6*
	10		10	7	0/7	+ 7.3/+8.1	1.72/2.88	40.2*
	25		10	7	0/7	+ 4.4/+8.1	1.40/2.88	51.4**

* $P<0.05$ for differences between inhibition due to combined drugs and TC-17.
** $P<0.05$ for differences between inhibition due to combined drugs and CPM.

on solid forms of Ehrlich carcinoma and sarcoma 180 is shown in Table 3. In a dose of 75 mg/kg, the activity increased with increasing numbers of injection and inhibition of 50% or more were observed with pretreatment of 3 injections in three days duration.

Effect of daily intraperitoneal injections of TC-17 for 21 days on solid tumors of Ehrlich carcinoma and sarcoma 180 is shown in Table 4. When the TC-17 treatment was performed after tumor inoculation, further repeated injections over a period of 7 days showed no essential change on tumor-inhibiting activity (see Table 1).

The potentiation of therapeutic effects of TC-17 by combinations with other anti-tumor agents is shown in Table 5. This experiment was carried

Table 6. Effect of TC-17 administered intraperitoneally on ascites tumors

Tumor	Dose (mg/kg/day)	Treatment (7 injections)	Mortality	Av. longevity *Ts/Cs**	ILS** (%)
Ehrlich carcinoma	75	Post-	5/5	10.4/11.8	−11.9
		Pre-	5/5	10.6/11.8	−10.4
Sarcoma 180	75	Post-	5/5	16.6/13.6	22.1
		Pre-	5/5	14.4/13.6	5.9
Yoshida sarcoma	100	Post-	7/7	12.8/14.0	− 8.6
		Pre-	7/7	11.4/14.0	−18.5

* The mean survival days of treated group/The mean survival days of control group
** Increase in life span : $(Ts/Cs-1)\times100$

out under the condition that the treatment of drug was initiated 24 hours after tumor inoculation. The combined administration of TC-17 with mitomycin C or cyclophosphamide resulted in inhibition of tumor growth that was significantly different from that obtained with the same doses of either drug alone. All mice used in this experiments were normal in behavior and appearance.

Table 6 shows the results of experiment on the ascites forms of mouse and rat tumors. Both post-treatment and pretreatment of TC-17 had little or no effect on the ascites forms.

Table 7. Acute toxicity of TC-17

	LD_{50} (mg/kg)			
	iv	ip	sc	po
Mouse (male)	145 (116～181)*	540 (461～637)	700 (583～840)	>2000
Rat (male)	500 (423～591)	560 (475～661)	—	—

* Confidence limits for p=95%

Table 8. Blood picture of rabbits treated with TC-17

Dose (mg/kg/day, iv)	No. of rabbits	No. of injections	Days after injections	Cell counts RBC(10^6)	WBC	Platelets(10^3)
25	4	21	0	5.97	5,600	290
			8	5.24	6,800	310
			15	5.14	9,600	400
			22	4.98	9,730	260
50	5	1	0	4.66	9,150	422
			3	5.34	10,380	410
			5	5.56	10,040	371
			10	5.21	9,010	322
			20	4.73	11,743	406
			30	5.21	9,280	388
	2	4	0	4.68	10,433	313
			8	4.61	10,533	383
			15	4.62	13,867	400
			20	4.55	10,330	453
			30	4.91	11,500	650

Toxicity :

Acute toxicity (LD_{50}) of TC-17 is shown in Table 7. The LD_{50} obtained in the various routes emphasized the low toxicity of TC-17 to mice and rats. Intraperitoneal administration of TC-17 produced symptoms of stretching with short duration and mild diarrhea in high dose, and intravenous administration caused only mild diarrhea.

Animals given TC-17 subcutaneously developed symptoms of fighting temporarily and in some cases produced a congestion and necrosis in the local region. In the case of oral administration, no changes of behavior were noted.

Blood Test :

Effect of TC-17 on peripheral blood picture is shown in Table 8. In groups being given 21 successive daily intravenous injections of TC-17 (25 mg/kg), a slight decreasing tendency of RBC

counts and an increase of WBC counts resulted. Single injection and 4 successive injections in dose of 50 mg/kg/day showed little or no changes of RBC, WBC and platelet counts. TC-17 did not cause a leucopenic action.

Discussion

TC-17, as demonstrated in the present paper, is a new compound which have unique biological activities. The tumor-inhibiting activity with pretreatment is more potent than that with treatment following the tumor inoculation on solid forms and its marked pretreatment activity continues for two weeks, probably because of its slow metabolism or elimination, but the compound has little or no effect on ascites forms. The repeated intravenous injections into the rabbits produce little or no hematological changes.

It has been reported[18] that pretreatment with some polysaccarides from higher plants is equally effective as treatment following the tumor implantation. In this case, it is suggested that the polysaccharides may be associated with the host defence mechanism to tumor growth. While, it has been known[19] that pretreatment with neoplastic agents (Cyclophosphamide, TEM, Hydrocortisone) which cause lymphopenia enhance the growth of intravenously injected Ehrlich ascites tumorc ells, probably due to a reduction in host resistance.

Mechanism of tumor-inhibiting activity of TC-17 is unknown at present but it seems to differ from the mode of action of polysaccharide, because of the result that decrease of tumor growth under the repeated injections of TC-17 for 3 weeks is approximately the same as that under injections for only 7 days.

From the results demonstrated in this paper, it is suggested that pretreatment of animals with TC-17 may be associated to interfere metastasis formation produced by disseminated tumor cells.

Further experimental studies of TC-17 on host animal and tumor cell are of much interest to know numerous informations on its biochemical and pharmacological properties.

Summary

Anti-tumor activity of TC-17 [4,4′-bis(2-*o*-oxyanilino-4-*m*-sulfoanilino-1, 3, 5-triazyl-6) stilbene-disulfonate-2,2′] derived from one of fluorescent dyes was investigated.

1. The tumor-inhibiting activity of TC-17 on solid forms of Ehrlich carcinoma and sarcoma 180 in mice was found to be very marked when the treatment was given parenterally prior to tumor inoculation, whereas the compound showed only moderate activity when the treatment was performed after tumor inoculation. TC-17 showed moderate effect with pretreatment on MH 134 hepatoma in mice and Yoshida sarcoma in rats, little or no effect on ascites tumors in mice and rats. When the compound was given orally, no tumor-inhibiting activities were observed.

2. The marked tumor-inhibiting activity of TC-17 with pretreatment continued for 1～2 weeks.

3. The combined administration of TC-17 with other anti-tumor agents resulted in synergistic inhibitory action of tumor growth without any undesirable effects.

4. TC-17 showed a weak toxicity in mice and rats, and significant changes on blood picture of rabbits were not observed.

Acknowledgments

The authors wish to thank Dr. S. YAMAOKA of this Pharmaceuticals Division for many helpful discussions and suggestions during this work and Mr. S. TANIDA and Miss F. MURAKAMI for technical assistance.

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