ANTITUMOR ACTIVITY OF TRIENOMYCIN A ON MURINE TUMORS

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The antitumor activity of a novel ansamycin antibiotic, trienomycin A, against various murine tumors was studied with two treatment schedules. The intraperitoneal injection of the antibiotic showed remarkable antitumor activity on sarcoma 180 and P388 leukemia at doses of 160 or 320 mg/kg, showing 151% and 100% increase in life span, respectively.

Trienomycin A inhibited the growth of Ehrlich and Meth A cells *in vitro* at doses of 0.1~0.4 μg/ml when the cells were exposed to the antibiotic for 72 hours. The incorporation of [^{3}H]thymidine into acid precipitable material in HeLa cells was slightly more marked than that of [^{3}H]uridine and [^{3}H]leucine when the cells were exposed to 0.04 or 0.08 μg/ml of trienomycin A for 4 hours. It appeared that trienomycin A showed antitumor activity by direct cytotoxic action.

Trienomycin A, a novel ansamycin antibiotic, was first reported by UMEZAWA *et al.* as a cytocidal antibiotic[1]. Trienomycin A is unique among the benzenoid ansamycin antibiotics in that it does not have a *p*-quinone or *p*-hydroquinone moiety in the structure[2]. The benzenoid moiety of trienomycin A is somewhat similar to that of maytansinoids derived from plant materials[3].

Thereafter, two other fractions, trienomycins B and C, were isolated from the same fermentation broth[4]. Among the trienomycins, substance A showed remarkable cytocidal activity against HeLa cells *in vitro*. The experiments reported herein were designed mainly to elucidate the antitumor activity of trienomycin A on transplantable murine tumors.

Materials and Methods

Animals

Female ICR, *dd*Y, CDF_1 and C57BL/6 mice, 6 weeks of age, were purchased from the Shizuoka Laboratory Animal Center (Hamamatsu, Japan).

Agents

Trienomycin A was isolated and purified according to the some procedure reported previously[1]. Trienomycin A was dissolved in a small amount of MeOH and Tween 80, diluted with saline and injected intraperitoneally into tumor bearing mice. [^{3}H]Thymidine([^{3}H]TdR, 22 Ci/mmol), [^{3}H]uridine ([^{3}H]UR, 6.5 Ci/mmol) and [^{3}H]leucine (60 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, UK.

Tumor Cell Lines and Antitumor Activity

Tumor cells were maintained by weekly passage intraperitoneally in mice. Tumor cell lines and mice used are listed in Table 1. Antitumor activity was evaluated by the increase in life span (ILS): $(T/C-1)\times 100(\%)$, where "T" is the mean survival days (MSD) of the treated group and "C" is the MSD of the control group. Survival of the mice was scored 60 days after inoculation of tumors,

Table 1. Tumors used in this experiment.

Tumor	Mice	Inoculum	
		Size (cells/mouse)	Site
Ehrlich carcinoma	*dd*Y	2.5×10^6	ip
Sarcoma 180	ICR	1×10^6	ip
IMC carcinoma	CDF_1	1×10^6	ip
P388 leukemia	CDF_1	1×10^5	ip
B16 melanoma	C57BL/6	3×10^5	ip
Lewis lung carcinoma	C57BL/6	3×10^5	ip

Table 2. Cytocidal activity of trienomycin A against Ehrlich and Meth A cells *in vitro*.

Concentration (μg/ml)	Ehrlich cells		Meth A cells	
	Cell No./plate ($\times10^4$)	Inhibition (%)	Cell No./plate ($\times10^4$)	Inhibition (%)
Control	88.1±15.9	0	43.0±1.9	0
1.6	6.5±2.2*	92.7	8.6±2.1**	80.0
0.4	19.7±4.1*	77.6	14.9±1.8**	65.3
0.1	66.4±4.5	24.5	33.6±5.8*	23.3
0.025	83.7±7.8	5.0	39.0±4.0	9.3
0.006	79.3±7.4	9.9	45.8±5.0	0

* $P<0.05$. ** $P<0.01$.

and mice remaining alive after this period of observation were considered cured.

Cytotoxicity for Tumor Cells

Ehrlich and Meth A tumor cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and kanamycin (50 μg/ml) at 37°C. To determine the cytotoxicity of trienomycin A, Ehrlich cells (7.5×10^4) or Meth A cells (3.8×10^4) in 1.5 ml of medium were placed in a tissue culture plate (Falcon, 24 wells) and incubated for 48 hours at 37°C in a 5-% CO_2 - 95% air atmosphere. To each culture well was added 0.5 ml of fresh medium containing a different concentration of trienomycin A, and they were reincubated. After further 72 hours of incubation the cells were counted by a hemocytometer.

Measurement of Macromolecular Synthesis

HeLa cells (5×10^4/0.2 ml) were plated on a cover slip (LUX, 13 mm i.d.) inserted previously into a tissue culture plate (Falcon, 24 wells), and 2 ml of minimum essential medium was added after 3 hours. Following 48 hours of incubation, each culture well was refilled with fresh medium containing a different concentration of the antibiotic. The cells were exposed to trienomycin A for 1 and 4 hours. One hour before termination of the culture, various precursors were added. At the end of the incubation period, cells were washed once with ice-cold phosphate buffered saline, and three times with ice-cold 5% TCA. The radioactivity of acid-precipitable material on the cover slip was measured by an Aloka liquid scintillation spectrometer.

Results

Cytotoxicity for Tumor Cells

Cytotoxic activity of trienomycin A was determined on Ehrlich carcinoma cells and Meth A fibrosarcoma cells *in vitro*. When the cells were exposed to trienomycin A for 72 hours, cell growth was remarkably suppressed at a dose of 0.4 μg/ml (Table 2).

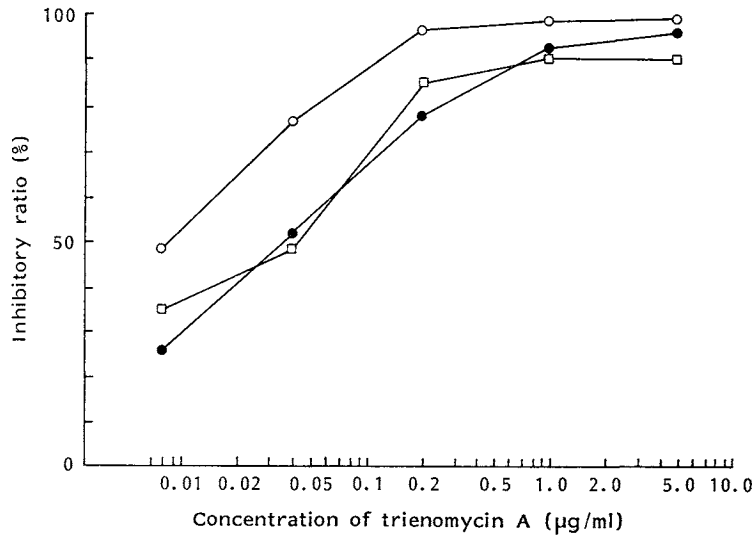
Table 3. Antitumor activity of trienomycin A.

Dose (mg/kg)	Sarcoma 180		P388 leukemia		IMC carcinoma	
	MSD	ILS (%)	MSD	ILS (%)	MSD	ILS (%)
Saline	10.0±0.8	0	10.4±0.5	0	16.6±2.6	0
32×10	22.8±3.9*	128	18.2±2.3*	75	27.4±4.3*	65
16×10	24.8±3.4*	148	17.0±2.2*	64	21.6±2.7*	30
8×10	22.4±5.9*	124	15.2±1.1*	46	20.2±1.6*	22
4×10	13.0±1.8*	30	13.6±0.5*	31	18.4±2.6	11
0.5×10 (MMC)	38.6±5.9*	286	23.0±4.0*	121	53.0±6.0*	219
Saline	11.8±0.8	0	10.2±0.4	0	14.5±1.0	0
64×5	5.8±0.8	−48	20.4±3.3*	100	5.4±0.9	−63
32×5	29.6±11.3*	151	17.8±0.4*	75	22.6±2.7*	56
16×5	25.6±7.8*	117	14.0±0.9*	37	19.2±1.3*	32
8×5	23.2±9.4*	97	13.7±0.8*	34	ND	

Dose (mg/kg)	B16 melanoma		Lewis lung carcinoma		Ehrlich carcinoma	
	MSD	ILS (%)	MSD	ILS (%)	MSD	ILS (%)
Saline	19.4±1.9	0	16.6±0.5	0	18.5±1.3	0
32×10	ND		ND		37.0±2.6*	100.0
16×10	22.2±0.4*	14.4	20.6±0.9*	24.0	25.6±7.2	38.4
8×10	21.0±2.3	8.2	19.2±2.2	15.7	18.3±1.5	0
4×10	22.0±1.4	13.4	16.4±3.1	0	18.8±1.5	1.6
0.5×10 (MMC)	23.0±2.0*	18.6	19.4±2.5	16.9	44.0±5.0*	138.0
Saline	21.0±1.2	0	22.5±0.5	0	19.4±1.3	0
32×5	26.8±3.5*	27.6	24.8±3.1	10.2	20.4±2.2	5.0
16×5	28.2±3.0*	34.3	20.2±2.2	0	20.8±1.5	7.0
8×5	29.2±3.8*	39.0	22.0±6.2	0	17.4±1.2	0

* $P<0.05$.
MMC: Mitomycin C.
ND: Not done.

Fig. 1. Effect of trienomycin A on the synthesis of macromolecules in HeLa cells.
○ Thymidine, ● uridine, □ leucine incorporation.



Antitumor Activity

Trienomycin A was evaluated for antitumor activity against murine tumors. According to the ILS of tumor bearing mice, trienomycin A was effective against sarcoma 180, IMC carcinoma, P388 leukemia and Ehrlich carcinoma at doses of 40~320 mg/kg. No definite difference was observed in the effect of trienomycin A between 5- and 10-day treatments on sarcoma 180, P388 leukemia and IMC carcinoma. Only a slight life prolongation effect was observed on Lewis lung carcinoma and B16 melanoma by the 10-day or the 5-day treatment schedules, respectively (Table 3).

Table 4. Antibacterial activity of trienomycin A.

Test organism	MIC (μg/ml)
Piricularia oryzae KF 180	100
Mucor racemosus KF 223 (IFO 4581)	>100
Saccharomyces sake KF 26	>100
Candida albicans KF 1	>100
Penicillium herquei KF 227 (IFO 4674)	100
Aspergillus niger KF 103 (ATCC 6275)	>100

MIC was determined by the conventional serial dilution method using potato - glucose agar.

Effect of Trienomycin A on Macromolecular Synthesis

The effect of trienomycin A on the incorporation of [^{3}H]TdR, [^{3}H]UR and [^{3}H]leucine into acid-precipitable macromolecules of HeLa cells was determined.

When HeLa cells were exposed to 0.008~0.2 μg/ml of the antibiotic for 4 hours, the incorporation of [^{3}H]TdR into acid precipitable material was slightly more marked than that of the other two precursors at lower concentrations of the antibiotic (Fig. 1).

Discussion

As shown in this study, trienomycin A possessed antitumor activity against murine tumors. The antibiotic also inhibited the growth of mammalian cells at doses of 0.4~0.1 μg/ml, whereas the growth inhibition of bacteria was weak as shown in the previous paper (paper-disk method)[1] and in Table 4 (agar dilution method). Among several ansamycin antibiotics, mycotrienins I and II are similar to trienomycin A in structure[5]. However, mycotrienins did not show definite anti-P388 activity *in vivo* in contrast to antifungal activity *in vitro*[6]. The structure of trienomycin A is unique among benzenoid ansamycin antibiotics in that the antibiotic does not have a *p*-quinone or *p*-hydroquinone moiety in its structure. This unique structure could be important because it possesses antitumor activity instead of antifungal activity. Cytotoxicity was decreased when the triene moiety of trienomycin A was reduced by catalytic hydrogenation (data not shown). This fact indicated that the triene moiety is essential for the activity of trienomycin A.

Inhibition of incorporation of [^{3}H]TdR into acid precipitable materials of mammalian cells was relatively more marked than that of the other two precursors. However, no definite characteristic was observed in the present brief experiment. We are now investigating the mechanism of action of this antibiotic, and the results will be reported elsewhere.

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