ANTITUMOR ACTIVITY OF ANKINOMYCIN

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As reported in our previous paper[1], a new antitumor antibiotic, named ankinomycin, was found in the culture broth of *Streptomyces* sp. SF2587. The antibiotic belongs to oxabenzanthraquinone antibiotics and has potent antitumor activity. In this paper, we describe the *in vitro* cytotoxicity and *in vivo* antitumor activity of ankinomycin against various type of tumors.

Six murine tumor cell lines and nineteen human tumor cell lines were used for the *in vitro* cytotoxicity test. The cytotoxicity test was carried out as follows: Cells were seeded to 96-well flat-bottomed microtiter plate (Falcon, No. 3002) 3,000 cells/well in 140 μl of RPMI-1640 medium containing 10% of fetal calf serum and 10 μM of 2-hydroxyethyldisulfide. After 24 hours incubation at 37°C in 5% CO_2, 20 μl of sample solution was added and then the mixture was further incubated for 72 hours. Surviving cells were counted by modified MTT assay[2,3] and 50% inhibitory concentration (IC_{50}) value was calculated by PROBIT's method[4]. The cytotoxicity of ankinomycin was compared with that of doxorubicin

Table 1. *In vitro* cytocidal activity of ankinomycin and doxorubicin.

Cell line		IC_{50} value (ng/ml)	
		Ankinomycin	Doxorubicin
Mouse			
P388	Leukemia	0.39	21
P388/ADR	Leukemia[a]	1.1	600
L1210	Leukemia	0.97	82
L1210/CPR	Leukemia	1.9	180
B16	Melanoma	1.7	52
Meth-A	Fibrosarcoma	1.9	200
Human			
K562	Leukemia (Myelocytic)	0.95	30
HL-60	Leukemia (Promyelocytic)	0.17	56
CCRF-CEM	Leukemia (T-cell)	0.53	46
MOLT-3	Leukemia (T-cell)	0.30	16
CCRF-SB	Leukemia (B-cell)	1.1	24
J-111	Leukemia (Monocytic)	0.67	50
KB	Nasopharynx carcinoma	3.0	98
PC-10	Lung carcinoma	1.2	250
PC-13	Lung carcinoma	2.6	87
PC-14	Lung carcinoma	5.0	360
MKN-1	Gastric carcinoma[b]	7.4	94
MKN-28	Gastric carcinoma[c]	2.9	150
MKN-45	Gastric carcinoma[d]	3.1	75
MKN-74	Gastric carcinoma[c]	2.4	60
YT/nu	Neuroblastoma	1.6	64
GOTO	Neuroblastoma	1.6	50
T-24	Urinary bladder carcinoma	1.8	65
HeLa S3	Uterine cervix carcinoma	4.4	120
HMV	Melanoma in vagina	0.22	80

Cells were incubated with each samples for 72 hours in RPMI-1640 medium supplemented with 10% of fetal calf serum and 10 μM of 2-hydroxyethyldisulfide. The rate of survival cells was measured with modified MTT assay and IC_{50} value was calculated with PROBIT's method.

[a] Multidrug-resistant subline of P388 leukemia. [b] Adenosquamous. [c] Well differentiated. [d] Poorly differentiated.

Table 2. Antitumor activity of ankinomycin towards various tumor lines.

Dose (mg/kg/day)	ILS (%)					
	P388[a]	P388/ADR[a]	L1210[b]	EL-4[a]	B16[c]	M5076[a]
0.4	39.1	−1.8	75.0	19.0	−51.3	−45.9
0.2	71.7	64.3	75.0	32.1	22.6	46.6
0.1	71.7	66.1	60.0	27.4	38.3	20.9
0.05	43.5	51.8	50.0	17.9	22.6	23.6
0.025	23.9	25.0	40.0	6.0	21.7	0.7
0.013	19.6	8.9	27.5	4.8	12.2	−4.1
0.0063	−2.2	3.6	ND	ND	ND	ND
Control (days)	9.2±0.84	11.2±0.45	8.0±0	16.8±0.45	23.0±3.39	29.6±1.52

Tumor cells were intraperitoneally implanted to each mouse and drug solution was intraperitoneally administered once a day at day-1, 4 and 7 ($n=5$).

[a] 1.0×10^6 cells/mouse were intraperitoneally implanted. [b] 1.0×10^5 cells/mouse were intraperitoneally implanted. [c] 0.5 ml of 10%-tumor brei was intraperitoneally implanted.

ILS: Increase in life span. ND: Not done.

Table 3. Antitumor activity of ankinomycin towards P388 leukemia by oral administration.

Dose (mg/kg)	ILS (%)	
	Oral	ip
6.4	−20	ND
3.2	46	ND
1.6	34	ND
0.8	12	ND
0.4	ND	12
0.2	ND	66
0.1	ND	42
0.05	ND	40
0.025	ND	24

1×10^6 cells/mouse of P388 cells were intraperitoneally implanted and ankinomycin solution was administered on day-1 only by oral or ip route ($n=5$).

ILS: Increase in life span. ND: Not done.

against various murine and human tumor cells (Table 1). Ankinomycin exhibited 13 to 550 times stronger cytotoxicity than doxorubicin against all cells examined in this study. Furthermore, ankinomycin exhibit strong cytotoxicity against P388/ADR, multidrug-resistant cells[5] and L1210/CPR, cisplatin-resistant cells.

We also studied the *in vivo* antitumor activity of ankinomycin against murine tumors. P388 leukemia, P388/ADR leukemia, L1210 leukemia, EL-4 lymphoma, B16 melanoma and M5076 ovarian carcinoma were used. Tumors cells were suspended in HANKS' solution and intraperitoneally implanted to BDF_1 male mice. Ankinomycin was dissolved in dimethyl sulfoxide and diluted with distilled water. The sample solution was administered intraperitoneally once a day at day-1, 4 and 7. As shown in Table 2, ankinomycin exhibited marked antitumor activity against P388, P388/ADR and L1210 and weak antitumor activity against EL-4, B16 and M5076. Furthermore, by oral administration, ankinomycin exhibited marked antitumor activity against P388 leukemia, as shown in Table 3.

As mentioned above, ankinomycin exhibited stronger cytotoxicity than doxorubicin and marked antitumor activity against several murine tumors including multidrug-resistant tumor. Therefore, ankinomycin appears to be a good candidate for useful antitumor drugs.

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