A NEW MACROMOLECULAR ANTITUMOR ANTIBIOTIC, C-1027

III. ANTITUMOR ACTIVITY

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C-1027, a new macromolecular antitumor antibiotic produced by *Streptomyces globisporus* C-1027, showed extremely potent cytotoxicity toward cultured cancer cells. Compared in terms of IC_{50} values, antibiotic C-1027 showed much more potent cytotoxicity than doxorubicin, mitomycin C and neocarzinostatin. Spermatogonial assay, a prescreen for anticancer drugs, was highly sensitive for detection of C-1027. At tolerable doses, C-1027 exhibited marked inhibition on a panel of transplantable tumors in mice, which included leukemia L1210, P388, ascites hepatoma H22, sarcoma 180 and melanoma Harding-Passey.

The new antitumor antibiotic C-1027 was discovered during antitumor screening of microbial metabolites by use of a spermatogonial assay, a new prescreen for detection of antitumor drugs[1], and was isolated from the culture filtrate of a new isolate identified as *Streptomyces globisporus* C-1027. The procedure for isolation and purification, as well as physico-chemical and biological properties, of C-1027 were reported previously[2,3]. Antibiotic C-1027, an acidic protein with a molecular weight of 15,000 daltons, had moderate antimicrobial activity against Gram-positive bacteria, but was shown to be inactive against Gram-negative bacteria except for some strains of *Escherichia coli* and fungi tested. Our studies have shown that antibiotic C-1027 is highly active in the spermatogonial assay, exhibits extremely potent cytotoxicity toward various human cancer cells *in vitro*, and displays inhibitory effect on a panel of transplantable tumors in mice. This paper reports data on the antitumor activity of antibiotic C-1027.

Materials and Methods

Antibiotics

Highly purified antibiotic C-1027 was prepared from a culture filtrate of *S. globisporus* C-1027 by a purification procedure improved over the previous one[3]. C-1027 was simply purified by ammonium sulfate precipitation at pH 4, followed by column chromatography on diethylaminoethyl (DEAE)-cellulose (Wako Pure Chemical Industries, Ltd.), and Butyl-Toyopearl 650 C (Tosoh Manufacturing Co., Ltd.) and by gel filtration chromatography on Sephadex G-50 (Pharmacia Fine Chemicals). Doxorubicin (ADR) and mitomycin C were purchased from Farmitalia, Italy, and Kyowa Hakko Kogyo Co., Ltd., Japan, respectively. Neocarzinostatin was manufactured by Yamanouchi Pharmaceutical Co., Ltd., Japan. Azaserine, sibiromycin and streptonigrin were isolated and purified at Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences.

Spermatogonial Assay

As described previously[1], male mice, weighing 22 to 28 g, were injected intratesticularly with

0.03 ml of the sample solutions for each testis. Three days after injection, mice were sacrificed and the testes were fixed in BOUIN's solution and embedded in paraffin. The histological sections, 5 μm in thickness, were stained with hematoxylin and eosin, and observed microscopically. Results were evaluated on the basis of specific disappearance of spermatogonia caused by the test materials. The criterion for a positive effect is that a marked decrease in number or complete disappearance of type B spermatogonia occurs, while other seminiferous cells remain unchanged.

Clonogenic Assay

Established human cancer cell lines of various origin including lung cancer A549, liver cancer BEL-7402, nasopharyngeal cancer CNE-2 and gastric cancer MGC-803 were used for the assay[4~6]. Cells were grown as monolayers in RPMI 1640 medium (Grand Island Biological Co., N.Y., U.S.A.) supplemented with 10% fetal calf serum with addition of benzylpenicillin (100 U/ml) and streptomycin (100 μg/ml) and incubated at 37°C in a 5% CO_2 - 95% air, humidified atmosphere. Logarithmically growing cells were seeded into 24-well plates with 100 cells/ml/well. After incubation of 24 hours, the spent medium was replaced with fresh medium and a 0.1-ml of various concentration of the desired test material was added into the well. Seven days after cell seeding, cell colonies were counted under an inverted microscope. Concentration for 50% colony inhibition (IC_{50}) of each drug was calculated.

Inhibition of Tumor Growth in Mice

Transplantable tumors including leukemia L1210, P388, ascites hepatoma H22, sarcoma 180, melanoma Harding-Passey and melanoma B16 were used. Leukemia L1210 and P388 were grown in DBA/2 mice; while melanoma B16 was passaged in BDF_1 mice. Sarcoma 180 was transplanted in ICR mice, and the other 2 tumors were in Kunming (KM) mice. Tumor inoculation, treatment schedule and route of drug administration are described separately under the tables in the Results section. Therapeutic effect of the drug was evaluated by survival time for leukemia and ascitic tumors, and by tumor weight for solid tumors. Student's t-test was used for statistical analysis.

Results

Activity in Spermatogonial Assay

As shown in Table 1, antibiotic C-1027 was the most active of all the antitumor antibiotics tested in the spermatogonial assay, with a minimal effective concentration (MEC) of 0.0039 μg/ml. The MEC values of the other antitumor antibiotics ranged from 2 to 8 μg/ml, with the exception of streptonigrin.

Cytotoxicity toward Cancer Cells

As determined by clonogenic assay, antibiotic C-1027 was extremely potent against cultured human cancer cells, the IC_{50} values ranging from 1.5×10^{-17} M to 3.1×10^{-16} M. Among the 4 cell lines tested, lung cancer A549 cells were the most sensitive to antibiotic C-1027. Compared on the basis of IC_{50} values, C-1027 showed much more potent cytotoxicity toward cancer cells than ADR, mitomycin C and neocazinostatin (Table 2), which have been used clinically.

Table 1. MEC of various antitumor antibiotics in the spermatogonial assay.

Antitumor antibiotic	MEC (μg/ml)
Actinomycin D	2
ADR	2
Azaserine	31
Bleomycin	62
Chromomycin A_3	8
Daunorubicin	4
Mitomycin C	2
Neocarzinostatin	8
Sibiromycin	8
Streptonigrin	0.5
C-1027	0.0039

Acute Toxicity

The LD_{50} values of antibiotic C-1027 in KM

Table 2. Cytotoxicity of C-1027 toward various human cancer cell lines.

Cell line	Cell origin	IC_{50} (M)			
		ADR	C-1027	Mitomycin C	Neocarzinostatin
A549	Lung carcinoma	1.7×10^{-9}	1.5×10^{-17}	8.0×10^{-9}	1.2×10^{-8}
BEL-7402	Liver carcinoma	1.8×10^{-9}	3.1×10^{-16}	ND	1.6×10^{-8}
CNE-2	Nasopharyngeal carcinoma	1.8×10^{-9}	2.2×10^{-16}	1.8×10^{-8}	1.3×10^{-8}
MGC-803	Gastric carcinoma	1.5×10^{-9}	2.0×10^{-16}	1.6×10^{-8}	1.3×10^{-8}

For the clonogenic assay, carcinoma cells were exposed to the drugs for 6 days.
ND: Not determined.

Table 3. Antitumor activity of C-1027 against leukemia and ascites hepatoma in mice.

Tumor	Schedule	Dose (mg/kg)	T/C (%)	Long-term survivor (60 days)
L1210 (ip-ip)	Day-1	0.05	359***	8/10
	Day-1	0.025	382***	10/10
	Day-1	0.0125	357***	9/10
	Day-1	0.0062	315**	5/10
	Day-1, 6	0.0125	382***	10/10
	Day-1, 6, 11	0.0125	382***	10/10
	Day-1, 2, 3, 4, 5	0.0125	317**	5/10
P388 (ip-ip)	Day-1	0.05	301**	4/10
	Day-1	0.025	337**	7/10
	Day-1	0.0125	341**	7/10
Hepatoma H22 (ip-ip)	Day-1	0.025	257**	
	Day-1	0.0125	320**	
	Day-1	0.0062	147**	

** $P<0.01$, *** $P<0.001$.

L1210, P388 leukemia and ascites hepatoma H22 were inoculated ip into ten mice per each groups on day-0 at inoculum size of 10^6 cells per mice, respectively. Drug was administered ip on the days indicated. The mean survival times of the control groups with L1210, P388 and hepatoma H22 were 15.7, 13.9 and 15.0 days, respectively. The antitumor activity was expressed as T/C values, T/C (%)=(mean survival days of drug-treated mice)/(mean survival days of control mice)×100.

mice were approximately 0.2 mg/kg (iv) and 0.05 mg/kg (ip), respectively. Lethal dose of C-1027 which was administered intraperitoneally caused peritonitis and subsequently fibrous adhesions of the viscera. Three to four days after intravenous injection of a lethal dose of antibiotic C-1027 in mice (0.5 mg/kg), hemopoietic cell numbers were markedly depressed in bone marrow and in the spleen. Thymocytes in the cortex of thymus were also decreased in number. No histopathological changes were found in the heart, lungs, liver and kidneys.

Inhibitory Effect on Leukemia and Ascites Tumors

Antibiotic C-1027 exhibited a marked antitumor effect against three ascites tumors, L1210, P388 and hepatoma H22, as shown in Table 3. The growth of leukemia L1210 was strongly inhibited and many long-term survivor mice were observed. All treatment schedules showed a similar antitumor effect. C-1027 was also highly effective against leukemia P388 and hepatoma H22.

Inhibitory Effect on Solid Tumors

Antibiotic C-1027 inhibited the growth of sarcoma 180. At the maximum tolerable dose (0.05 mg/kg, iv), the inhibition level reached 53%, being comparable to that of ADR, as shown in Table 4.

Table 4. Antitumor activity of C-1027 against solid tumors in mice.

Drug	Tumor	Schedule	Dose (mg/kg)	Mortality	Inhibition (%)
C-1027	Sarcoma 180 (sc-iv)	Day-1, 5, 9, 13	0.1	2/7	(87)***
		Day-1, 5, 9, 13	0.05	0/7	53***
		Day-1, 5, 9, 13	0.025	0/7	25
		Day-1, 5, 9, 13	0.0125	0/7	16
		Day-1, 5, 9, 13	0.0063	0/7	10
ADR	Sarcoma 180 (sc-iv)	Day-1, 5, 9, 13	4.0	0/7	55***
		Day-1, 5, 9, 13	2.0	0/7	36***
C-1027	Melanoma	Day-1	0.1	0/10	81***
	Harding-Passey	Day-1	0.05	0/10	78***
	(sc-iv)	Day-1	0.025	0/10	49*
		Day-1, 4, 7	0.05	0/10	82***
C-1027	Melanoma B16 (sc-iv)	Day-1	0.1	0/10	9
		Day-1	0.05	0/10	0
		Day-1, 4, 7	0.1	0/10	44**
		Day-1, 4, 7	0.05	0/10	36**

* $P<0.05$, ** $P<0.01$, *** $P<0.001$.

For sarcoma 180, 10^6 cells were inoculated sc into each mouse. For melanoma Harding-Passey and melanoma B16, tumor cell suspension was prepared with tissue grinder by adding 1 part of tumor tissue into 5 parts of normal saline (w/v) and 0.2 ml of suspension was inoculated sc in the axilla region of each mouse on day-0. Drug was administered iv on the days indicated. The day of evaluation for sarcoma 180 and melanoma Harding-Passey and melanoma B16 was the 15th, 20th and 14th day, respectively. The mean tumor weights of control groups with respective tumors were 10.9, 6.2 and 6.7 g.

C-1027 was also highly effective against melanoma Harding-Passey, showing a growth inhibitory effect of 78~81% by a single iv injection at tolerable doses. C-1027 had a moderate, positive effect on melanoma B16.

Discussion

The present study provides evidence that antibiotic C-1027 has extremely potent cytotoxicity against cancer cells *in vitro* and is effective against a panel of transplantable tumors in mice at tolerable doses. In comparison with neocarzinostatin, a known macromolecular peptide antitumor antibiotic, C-1027 shows more potent cytotoxicity toward cancer cells, with IC_{50} values much lower than the picogram level. The cytotoxicity of C-1027 is also much stronger than that of other macromolecular peptide antibiotics such as macromomycin[7] and AN-7[8]. In recent years, several highly potent novel antibiotics which include CC-1065[9], FR-900405 analogs[10], PD 114,759 analogs[11], esperamicins[12] and calichemicins[13,14] have been reported. However, those antitumor antibiotics are not macromolecular peptides. To our knowledge, antibiotic C-1027 is the most potent of the macromolecular peptide antitumor antibiotics ever reported.

In the course of screening for new antitumor antibiotics, we employed the spermatogonial assay as a prescreen to examine fermentation liquors, and C-1027 was one of the positives from approximately 2,000 samples screened. The present studies show that the highly purified C-1027 is the most active of all antitumor antibiotics tested in the spermatogonial assay. It appears that this assay may be useful for detection of highly potent antitumor antibiotics.

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