A NEW ANTITUMOR ANTIBIOTIC, FR900840

III. ANTITUMOR ACTIVITY AGAINST EXPERIMENTAL TUMORS

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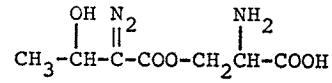
FR900840 ((2*S*)-2-amino-2-carboxyethyl (3*R*)-2-diazo-3-hydroxybutyrate), a new antibiotic with antitumor activity was isolated from the fermentation broth of *Streptomyces* sp. No. 8727. Its antitumor activity was examined in three mouse tumor systems and ten human tumor systems. FR900840 had no clear effect on mouse ascitic tumors, P388 and L1210, and the B16 melanoma line, but had prominent antitumor effects on several human solid tumors. Its antitumor activity against A549 human lung adenocarcinoma was stronger than those of vinblastine, doxorubicin and cisplatin. These results suggest that FR900840 may become a useful prototype antitumor drug.

A new antibiotic, FR900840, which was isolated[1] from *Streptomyces* sp. No. 8727 has the chemical structure of (2*S*)-2-amino-2-carboxyethyl (3*R*)-2-diazo-3-hydroxybutyrate (Fig. 1)[2]. It was shown to have potent antitumor effects *in vitro* and *in vivo* on some tumor systems.

Fig. 1. Structure of FR900840.



We report here the effects of FR900840 on the growth of three implanted mouse tumors, the growth of ten human tumors xenografted to nude mice, and the comparison of the antitumor effects with those of clinically used three antitumor drugs.

Materials and Methods

Drugs

FR900840 was prepared in our Research Laboratories. Vinblastine was purchased from Kyorin Pharmaceutical Co., Ltd. Doxorubicin was purchased from Kyowa Hakko Kogyo Co., Ltd. Cisplatin was purchased from Nippon Kayaku Co., Ltd. FR900840 was dissolved in or diluted with phosphate buffered saline (NaCl 80 g, KCl 2 g, Na_2HPO_3 2 g per liter; pH 7.2, PBS (−)) and other drugs were dissolved in or diluted with saline just before use. The solutions were given ip or iv at a volume of 10 ml/kg. PBS (−) or saline was given to the control mice.

Animals

Female mice of DBA/2, C57BL/6, BDF_1 (C57BL/6×DBA/2), Jcl:AF-nu strains and both sex mice of BALB/C nu/nu strain were purchased from Charles River Japan Inc., Atsugi-City, Japan. Six to tenweek-old mice were used throughout the experiments.

Tumors

Mouse leukemia P388 and L1210 were maintained ip by serial passage in DBA/2 mice. Mouse

Table 1. Tumors, mice and injection methods.

Tumor system			Mouse		Drug	
Tumor	Site	Tissue	Strain	Sex[a]	Route	Schedule[b]
Mouse tumors						
P388 Leukemia	ip	1×10^6 cells	BDF_1	F	ip	QD D1-4
L1210 Leukemia	ip	1×10^5 cells	BDF_1	F	ip	QD D1-4
B16 Melanoma	ip	1:10 brei (0.5 ml)	BDF_1	F	ip	QD D1-4 and D7-10
Human tumors						
A549 Lung adenocarcinoma	SRC	Fragment ($1\times1\times1$ mm)	BDF_1	F	ip	Q4D D1, 5, 9
	sc	Fragment ($2\times2\times2$ mm)	BALB/C nu/nu	F	iv	Q4D D24, 28, 32
L27 Lung adenocarcinoma	sc	Fragment ($2\times2\times2$ mm)	BALB/C nu/nu	M	iv	Q4D D14, 18, 22
LC-6 Lung squamous cell carcinoma	sc	Fragment ($2\times2\times2$ mm)	BALB/C nu/nu	M	iv	Q4D D11, 15, 19
HLC Lung squamous cell carcinoma	sc	Fragment ($2\times2\times2$ mm)	BALB/C nu/nu	M	iv	Q4D D15, 19, 23
LX-1 Lung small cell carcinoma	sc	Fragment ($2\times2\times2$ mm)	BALB/C nu/nu	M	iv	Q4D D7, 11, 15
ADH Lung small cell carcinoma	sc	Fragment ($2\times2\times2$ mm)	BALB/C nu/nu	F	iv	Q4D D22, 26, 30
SC-6 Stomach adenocarcinoma	sc	Fragment ($2\times2\times2$ mm)	BALB/C nu/nu	M	iv	Q4D D13, 17, 21
MKN-74 Stomach adenocarcinoma	sc	Fragment ($2\times2\times2$ mm)	BALB/C nu/nu	F	iv	Q4D D19, 23, 27
ZR-75-1 Mammary adenocarcinoma	sc	Fragment ($2\times2\times2$ mm)	BALB/C nu/nu	F	iv	Q4D D8, 12, 16
MCF-7 Mammary adenocarcinoma	sc	Fragment ($2\times2\times2$ mm)	Jcl:AF-nu	F	iv	Q4D D21, 25, 29

[a] F: Female, M: male.
[b] D: Day.

melanoma B16 was maintained ip by serial passage in C57BL/6 mice. Used human tumor lines are listed in Table 1. A549, L27, LC-6, HLC, LX-1, ADH, SC-6 and ZR-75-1 were maintained sc by serial passage in BALB/C nu/nu mice. MCF-7 was maintained sc by serial passage in Jcl:AF-nu mice with the injection of E.P. Hormone Depo. (containing hydroxyprogesterone 50 mg/ml and estradiol 1 mg/ml: Teikoku Zoki Co., Ltd.) 0.06 ml/mouse intramuscularly every week.

The strain of mouse, inoculum site and size, route and schedule of drug injection used for drug evaluation tests are listed in Table 1.

Subrenal Capsule (SRC) Assay

We established a 2-week SRC assay by using the immunosuppressive agent FK-506 which was developed in our Research Laboratories[3,4]. The method of implanting tumors under the kidney capsule of the mouse was described by BOGDEN *et al.*[5,6]. A 1-mm^3 tumor fragment was implanted at day-0 and at day-14 mice were sacrificed, the tumor bearing kidney was exteriorized and the final size of the implanted tumor was measured. FK-506 (32 mg/kg) was injected sc at day-1, 2, 5, 7, 9 and 12 which completely inhibited the hosts immune response for rejecting the xenograft[4].

Evaluation of Antitumor Activity

Drug efficacy against mouse tumors assessed as percent of median survival time, was calculated by the following formula:

$$T/C\,(\%)=\frac{\text{Median survival time of the treated group}}{\text{Median survival time of the control group}}$$

Tumor volume was calculated by the following formula:

$$\text{Tumor volume}\,(mm^3)=1/2\times a\times b^2$$

The length (a) and width (b) of the tumor were measured by calipers.

In the experiments on human solid tumors, initial and final tumor volumes were calculated on the first injection day and the evaluation day (14 days from the first injection day), respectively. Relative mean tumor volume was calculated by the following formula:

$$\text{Relative mean tumor volume}=\frac{\text{Mean tumor volume (final)}}{\text{Mean tumor volume (initial)}}$$

Drug efficacy was expressed as percent of mean tumor volume of the treated group (T) to that of the control group (C).

$$\text{Growth inhibition}\,(\%)=(1-T/C)\times 100$$

Results

Antitumor Activity against Mouse Ascitic Tumors

The antitumor activity of FR900840 against three kinds of mouse ascitic tumors are shown in Table 2. FR900840 had no effect.

Table 2. Antitumor activities of FR900840 against mouse ascitic tumors.

Dose (mg/kg)	T/C (%)[a]		
	P388	L1210	B16
0.1			100
0.32	100	94	106
1	100	94	103
3.2	100	94	109
10	105	106	124
32	45 (Tox)[b]	106	124
100	45 (Tox)	65 (Tox)	

[a] Median survival times of control group with P388, L1210 and B16 were 11, 8.5 and 17 days, respectively. Twenty and 10 mice were used in control and drug treated groups respectively.

[b] Toxicity (Tox) indicates T/C (%)$\leqq$85.

Antitumor Activity against A549 Human Lung Adenocarcinoma in the SRC Assay

The antitumor activities of FR900840 and three clinically used antitumor drugs were examined in the SRC assay against A549 human lung adenocarcinoma. As shown in Table 3,

Table 3. Antitumor activities of several drugs against A549 (SRC assay).

Dose (mg/kg)	Growth inhibition (%)[a]			
	FR900840	Vinblastine	Doxorubicin	Cisplatin
0.01		4	10	−12
0.032		28	−3	−21
0.1	5	14	3	−13
0.32	20	38	19	−12
1	22	68 (7)	45	7
3.2	50	Tox (0)	51	14 (7)
10	56		Tox (1)	Tox (2)
32	88 (7)[b]			
100	Tox[c](4)			

[a] A 1-mm³ tumor fragment was implanted in the subrenal capsule at day-0 and drugs were given ip at day-1, 4 and 9. The final tumor size was measured at day-14. Eight mice were used in each group.
[b] Number in parentheses indicate the number of survivors on day-14.
[c] Toxicity (Tox) indicates survivor (%) on day-14 ≦65.

Table 4. Antitumor activities of FR900840 against subcutaneously-implanted human tumor xenografts in nude mice.

Dose (mg/kg)	Growth inhibition[a] (%)									
	Lung adenocarcinoma		Lung squamous cell carcinoma		Lung small cell carcinoma		Stomach adenocarcinoma		Mammary adenocarcinoma	
	A549	L27	LC-6	HLC	LX-1	ADH	SC-6	MKN-74	ZR-75-1	MCF-7
10	29		50	−33	−6		19		31	40
18		6	89		16	−2		26	38	40
32	41	48	99	16	6	24	48	41	37	47
56		20	100	31	18	29	66	49	44	51
100	60	52	100	42	17	Tox[b]	91	57	67	Tox

[a] Human tumor cells were implanted sc in nude mice on day-0. When the tumor volumes become 100~300 mm³ the drug was given iv 3 times every 4 days. Final tumor size were measured after 2 weeks from first drug injection. Five or 6 mice were used in each group. Jcl:AF-nu mice were used in MCF-7 and BALB/C nu/nu mice were used in other tumor systems.
[b] Toxicity (Tox) indicates survivor (%) on final day ≦65.

FR900840 exhibited prominent antitumor activity. Vinblastine and doxorubicin also inhibited the growth of A549 dose dependently, their activities were weeker than that of FR900840, however. Cisplatin had no clear antitumor effect on A549.

Activity against Human Xenograft Tumors

The experiments were performed to evaluate the antitumor activity of FR900840 against 10 kinds of human tumors, lung adenocarcinoma (A549 and L27), lung squamous cell carcinoma (LC-6 and HLC), lung small cell carcinoma (LX-1 and ADH), stomach adenocarcinoma (SC-6 and MKN-74), mammary adenocarcinoma (ZR-75-1 and MCF-7). The results are shown in Table 4. FR900840 had strong antitumor activity against LC-6 and SC-6, it also inhibited the growth of A549, L27, HLC, MKN-74, ZR-75-1 and MCF-7 in a dose dependent manner. It showed no clear antitumor activities against the lung small cell carcinomas LX-1 and ADH.

Discussion

FR900840 was found through an *in vitro* screening system using human tumor cell lines. It

inhibited the growth of human lung adenocarcinoma A549 and human mammary adenocarcinoma MCF-7, and the IC_{50} values were 0.50 and 0.55 μg/ml, respectively[1]. However, the IC_{50} value of the drug against mouse leukemia P388 *in vitro* was 33 μg/ml[1]. According to these data FR900840 was appeared to be a new type of antitumor drug. Therefore we examined the antitumor activity of the drug against several other mouse and human tumor systems.

As shown in Table 2, FR900840 had no clear antitumor activity against three mouse ascitic tumors. However, it showed prominent antitumor effects on several human solid tumors, lung adenocarcinoma, lung squamous cell carcinoma, stomach adenocarcinoma and mammary adenocarcinoma (Table 4). In the SRC assay system, its antitumor activity against A549 lung adenocarcinoma was stronger than those of vinblastine, doxorubicin and cisplatin (Table 3).

The use of *in vivo* murine leukemia models for large scale antitumor drug screening has been successful in identifying compounds with clinical activity[7~9]. For the most part, however, this clinical activity has been limited to leukemias, lymphomas and some rare solid tumors with very high growth rates. As shown in this report, FR900840 has no antitumor activity against murine leukemias but has prominent antitumor activity against most human solid tumors tested. We therefore expect FR900840 will become a useful prototype drug for human solid tumors.

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