A NEW ANTITUMOR ANTIBIOTIC, FR-900482

III. ANTITUMOR ACTIVITY IN TRANSPLANTABLE EXPERIMENTAL TUMORS

KYOICHI SHIMOMURA, OSAMU HIRAI, TAMOTSU MIZOTA, SANAE MATSUMOTO, JO MORI, FUMIO SHIBAYAMA and HIROYUKI KIKUCHI

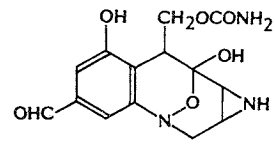
Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6 Kashima, Yodogawa-ku, Osaka 532, Japan

(Received for publication November 7, 1986)

FR-900482 (4-formyl-6,9-dihydroxy-14-oxa-1,11-diazatetracyclo[7.4.1.$O^{2,7}$.$O^{10,12}$]tetradeca-2,4,6-triene-8-ylmethyl carbamate), a new antibiotic with antitumor activity was isolated from fermentation broth of *Streptomyces sandaensis*. Its antitumor activities were studied and compared with that of mitomycin C (MMC) in animals. FR-900482 in doses of 0.32~10 mg/kg (ip) prolonged the life of mice bearing ascitic P388, L1210, B16, MM46, Ehrlich or EL4 tumors and rats bearing ascitic AH130 or AMC60 tumors. FR-900482 in doses of 5.6~18 mg/kg (iv) inhibited human LX-1, MX-1, SC-6 and LC-6 tumors xenografted sc in nude mice. FR-900482 was more effective than or equally effective to MMC in all the tumors used. FR-900482 was ineffective against cyclophosphamide-resistant P388, but was effective against MMC- or vincristine-resistant P388. The results suggest that FR-900482 may have clinical potential.

The new antibiotic, FR-900482, found and isolated in our Research Laboratories from *Streptomyces sandaensis* No. 6897[1], has a unique chemical structure of 4-formyl-6,9-dihydroxy-14-oxa-1,11-diazatetracyclo[7.4.1.$O^{2,7}$.$O^{10,12}$]tetradeca-2,4,6-triene-8-ylmethyl carbamate (Fig. 1)[2]. FR-900482 was shown to have good antitumor effects in transplantable tumor systems.

Fig. 1. Structure of FR-900482.



In this paper, we describe the antitumor activities of FR-900482 on various implanted mouse and rat tumors, and on human tumors xenografted to nude mice, and compare the effects with those of mitomycin C (MMC).

Materials and Methods

Drugs

FR-900482 was prepared in our Research Laboratories. MMC was purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo. Cyclophosphamide (CPM) and vincristine (VCR) were purchased from Shionogi & Co., Ltd., Osaka. The 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 body weight. Saline was given to the control animals.

Animals

Female mice of BDF_1 (C57BL/6×DBA/2), CDF_1 (BALB/c×DBA/2), DBA/2, C57BL/6, C3H/HeN and ICR strains, and male mice of BALB/c nu/nu strain were purchased from Charles River Japan Inc., Atsugi. Female rats of Donryu and ACI/N strains were purchased from Shizuoka Agri-

cultural Cooperative Association for Experimental Animals, Hamamatsu, and Hoshino Lab Animals, Yashio, respectively.

Tumors

P388 and L1210 leukemia were maintained ip by serial passage in DBA/2 mice. B16 melanoma (B16) was maintained ip by serial passage in C57BL/6 mice. MM46 mammary carcinoma (MM46) was maintained ip by serial passage in C3H/HeN mice. Ehrlich carcinoma (Ehrlich) was maintained ip by serial passage in ICR mice. EL4 lymphoma (EL4) was maintained ip by serial passage in C57BL/6 mice. P388/MMC, P388/CPM and P388/VCR leukemias (P388/MMC, P388/CPM and P388/VCR) were maintained ip by serial passage in CDF_1 mice. AH130 hepatoma (AH130) and AMC60 fibrosarcoma (AMC60) were maintained ip by serial passage in Donryu and ACI/N rats, respectively. Human LX-1 and LC-6-JCK lung carcinomas (LX-1 and LC-6-JCK), MX-1 mammary carcinoma (MX-1), and SC-6-JCK stomach carcinoma (SC-6-JCK) were maintained sc by serial passage in BALB/c nu/nu mice. The species and strain of animals, inoculum site and size, and route and schedule of drug injection used for drug evaluation tests are listed in Table 1.

Evaluation of Antitumor Activity

Drug efficacy against the ascitic tumors was assessed as percent of median (mean) survival time of the treated group (T) to that of the control group (C).

$$\text{T/C}\,(\%)=\frac{\text{Median (mean) survival time of (T)}}{\text{Median (mean) survival time of (C)}}\times 100$$

Tumor weight, as derived from caliper measurements of the length and width of tumors, was calculated by the formula: Tumor weight (mg)$=1/2\times a\times b^2$, where a represents the length and b represents the width (mm).

In the experiments on human solid tumors, initial and final tumor weights were calculated on the first injection day (just before dosing) and the last evaluation day, respectively. Relative mean tumor weight are shown for each group of mice.

Relative mean tumor weight=Mean tumor weight (final)/Mean tumor weight (initial)

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

$$\text{Growth inhibition}\,(\%)=\left(1-\frac{\text{Relative mean tumor weight (T)}}{\text{Relative mean tumor weight (C)}}\right)\times 100$$

Results

Antitumor Activity Against Ascitic Tumors in Mice and Rats

The antitumor activity of FR-900482 against 6 kinds of mouse ascitic tumors is shown in Tables 2 and 3. The tumors were inoculated ip to mice on day 0. In the experiment reported in Table 2, the drugs were given ip to mice once a day for 5 days (days 1~5) in the test on P388, and once a day for 9 days (days 1~9) in the tests on L1210 and B16. Both drugs dose dependently prolonged the life of mice bearing P388; the highest T/Cs were 305% for FR-900482 and 200% for MMC. Against L1210, FR-900482 showed antitumor activity at doses of 3.2 and 10 mg/kg but MMC was active only at 1.0 mg/kg. FR-900482 was more active than MMC against B16.

In the experiments reported in Table 3, the drugs were given ip to mice once a day on days 1, 5 and 9. FR-900482 was potently active against the 3 kinds of tumors tested, with antitumor effects comparable to those of MMC against MM46 and Ehrlich. The drug was more active than MMC against EL4.

Table 4 shows the antitumor activities of FR-900482 and MMC against rat ascitic tumors AH130

Table 1. Tumors, animals and injection methods.

Tumor system			Host				Drug	
Tumor	Site	Tissue	Strain	Age (weeks)	Sex	Body weight (g)	Route	Schedule
P388 leukemia	ip	1×10^6 cells	BDF_1	7	F	18.0~21.6	ip	QD D1~5
L1210 leukemia	ip	1×10^5 cells	BDF_1	7	F	17.8~22.0	ip	QD D1~9
B16 melanoma	ip	1: 10 brei (0.5 ml)	BDF_1	8	F	17.0~20.9	ip	QD D1~9
MM46 mammary carcinoma	ip	1×10^6 cells	C3H/HeN	7	F	20.4~24.8	ip	Q4D D1, 5 and 9
Ehrlich carcinoma	ip	1×10^6 cells	ICR	9	F	27.6~32.6	ip	Q4D D1, 5 and 9
EL4 lymphoma	ip	1×10^6 cells	BDF_1	7	F	18.3~22.9	ip	Q4D D1, 5 and 9
AH130 hepatoma	ip	1×10^6 cells	Donryu[a]	8	F	185~226	ip	Q4D D1, 5 and 9
AMC60 fibrosarcoma	ip	1×10^6 cells	ACI/N[a]	7	F	140~175	ip	Q4D D1, 5 and 9
LX-1 lung carcinoma	sc	Fragment (2×2×2 mm)	BALB/c nu/nu	5	M	18.7~25.5	iv	Q4D D12, 16 and 20
MX-1 mammary carcinoma	sc	Fragment (2×2×2 mm)	BALB/c nu/nu	6	M	19.4~22.7	iv	Q4D D11, 15 and 19
SC-6-JCK stomach carcinoma	sc	Fragment (2×2×2 mm)	BALB/c nu/nu	5	M	18.7~28.0	iv	Q4D D12, 16 and 20
LC-6-JCK lung carcinoma	sc	Fragment (2×2×2 mm)	BALB/c nu/nu	5	M	21.8~28.3	iv	Q4D D12, 16 and 20
P388/MMC, P388/CPM, P388/VCR, P388/S leukemia[b]	ip	1×10^6 cells	CDF_1	7~10	F	18.8~24.0	ip	QD D1

[a] Rats were used.
[b] Mitomycin C-resistant P388, cyclophosphamide-resistant P388, vincristine-resistant P388, non-resistant P388.

Table 2. Antitumor effects of FR-900482 against murine ascitic tumors.

Drug	Dose (mg/kg)	P388 (ip-ip) QD D1~5 T/C (%)	L1210 (ip-ip) QD D1~9 T/C (%)	B16 (ip-ip) QD D1~9 T/C (%)
FR-900482	0.1	105	100	115
	0.32	130	101	123
	1.0	160	119	162
	3.2	210	141	200
	10.0	305 (2)	190 (2)	350 (3)
MMC	0.032	120	105	139
	0.1	135	107	104
	0.32	150	117	150
	1.0	200	153	223
	3.2	80	90	62

Tumor cells were inoculated ip to mice on day 0 and drugs were given ip to the mice. Median survival times in mice bearing P388 and B16, and mean survival times in mice bearing L1210 were measured. Median survival times of control groups with P388 and B16 were 10 and 13 days, respectively. Mean survival time of control group with L1210 was 8.1 days. Twelve and 6 mice were used in control and drug treated groups, respectively, in P388 test. Twenty and 10 mice were used in control and drug treated groups, respectively, in L1210 and B16 tests. Numbers in parentheses indicate the number of survivors on day 36 in P388 test, on day 31 in L1210 test and on day 60 in B16 test.

Table 3. Antitumor effects of FR-900482 against murine ascitic tumors.

Drug	Dose (mg/kg)	MM46 (ip-ip) Q4D D1, 5 and 9 T/C (%)	Ehrlich (ip-ip) Q4D D1, 5 and 9 T/C (%)	EL4 (ip-ip) Q4D D1, 5 and 9 T/C (%)
FR-900482	0.32	98	105	96
	1.0	118 (2)	143 (4)	108
	3.2	>300 (9)	>270 (10)	135
	10.0	>300 (7)	>270 (6)	169 (3)
MMC	0.1	105 (2)	108	92
	0.32	>300 (9)	>149 (3)	85
	1.0	>300 (9)	>270 (7)	108
	3.2	195 (2)	>270 (9)	135

Tumor cells were inoculated ip to mice on day 0 and drugs were given ip to the mice. Median survival times in mice bearing tumors were measured. Median survival times of control groups were as follows: MM46; 20 days, Ehrlich; 18.5 days and EL4; 13 days. Twenty and 10 mice were used in control and drug treated groups, respectively. Numbers in parentheses indicate the number of survivors on day 60 in MM46 test, on day 50 in Ehrlich test and on day 40 in EL4 test.

and AMC60. The drugs were given ip on days 1, 5 and 9, and potently prolonged the life of rats bearing either kind of tumors (ip). Ninety percent of rats bearing AH130 were cured by treatment with either drug, and 4 of 7 and 2 of 7 rats bearing AMC60 were cured by treatment with FR-900482 and MMC, respectively.

Antitumor Activity Against Human Xenograft Tumor

The experiments were performed to evaluate the antitumor activities of FR-900482 and MMC against 4 kinds of human tumors, LX-1, MX-1, SC-6-JCK and LC-6-JCK, implanted sc in BALB/c nu/nu mice. Tumor weights were measured on day 28 in the experiments on LX-1, SC-6-JCK and LC-6-JCK, and on day 27 in the experiment on MX-1. As shown in Table 5, both drugs inhibited growth of all the tumors tested; weights of all tumors decreased by more than 90%.

Table 4. Antitumor effects of FR-900482 against rat ascitic tumors.

Drug	Dose (mg/kg)	AH130 (ip-ip) Q4D D1, 5 and 9 T/C (%)	AMC60 (ip-ip) Q4D D1, 5 and 9 T/C (%)
FR-900482	0.32	NT	100
	1.0	>429 (9)	189 (2)
	3.2	≧393 (5)	>333 (4)
	10.0	Toxic	—
MMC	0.1	>429 (9)	172 (1)
	0.32	>429 (9)	300 (2)
	1.0	>429 (9)	183 (2)

Tumor cells were inoculated ip to rats on day 0 and drugs were given ip to the rats. Median survival times in rats bearing tumors were measured. Median survival times of control groups with AH130 and AMC60 were 14 and 18 days, respectively. Ten and 7 rats were used per group in AH130 and AMC60 tests, respectively. Numbers in parentheses indicate the number of survivors on day 60.

Toxic: <50% survivors on the final evaluation day indicates toxicity.

NT: Not tested.

Table 5. Antitumor effects of FR-900482 against subcutaneously-implanted human tumor xenografts in nude mice.

Drug	Dose (mg/kg)	LX-1 (sc-iv) Q4D D12, 16 and 20 Growth inhibition (%)	MX-1 (sc-iv) Q4D D11, 15 and 19 Growth inhibition (%)	SC-6 (sc-iv) Q4D D12, 16 and 20 Growth inhibition (%)	LC-6 (sc-iv) Q4D D12, 16 and 20 Growth inhibition (%)
FR-900482	5.6	47	99	86	96
	10.0	57	100	99	100
	18.0	97	100	100	100
MMC	1.8	63	100	96	97
	3.2	98	100	99	99
	5.6	Toxic	Toxic	Toxic	Toxic

Human tumor cells were implanted sc in BALB/c nu/nu mice on day 0 and drugs were given iv to the mice. Tumor sizes were measured on day 28 in LX-1, SC-6-JCK and LC-6-JCK tests, and on day 27 in MX-1 test, and mean tumor weights were calculated. Mean tumor weights of control group were as follows: LX-1; 1,618±194 mg, MX-1; 732±48 mg, SC-6-JCK; 2,423±487 mg, and LC-6-JCK; 1,573±170 mg (±SE). Ten and 5 mice were used in control and drug treated groups, respectively, in LX-1 and MX-1 tests. Five mice were used in each group in SC-6-JCK test. Four and 5 mice were used in control and drug treated groups, respectively, in LC-6-JCK test.

Toxic: See Table 4.

Antitumor Activity Against Drug-resistant P388

This experiment was performed to examine FR-900482 for cross resistance to other chemotherapeutic drugs for antitumor activities. The results are shown in Table 6. FR-900482 was active against P388 which is either sensitive or resistant to MMC, whereas MMC was active against MMC sensitive P388 but not against MMC-resistant P388. The life of mice bearing CPM-resistant P388 was not prolonged significantly by FR-900482, MMC or CPM. FR-900482 and MMC were effective in VCR-resistant P388 model.

Discussion

Such tumors as P388, L1210, B16, LX-1 and MX-1 are widely used as models by the National Cancer Institute (NCI) and are understood to give good correlation between animal and clinical effects

Table 6. Antitumor effects of FR-900482 against drug-resistant P388 leukemia.

Drug	Dose (mg/kg)	P388/MMC T/C (%)	P388/S T/C (%)	P388/CPM T/C (%)	P388/S T/C (%)	P388/VCR T/C (%)	P388/S T/C (%)
FR-900482	1	109	120	89	120	140	135
	3.2	141	120	94	—	170	—
	10	141	160	117	170	200	180
	18	155	185	—	—	—	—
MMC	1	109	120	100	140	160	140
	3.2	114	150	111	160	190	155
	5.6	109	180	122	—	210	—
Cyclo-	56			94	—		
phosphamide	100			94	245		
	180			89	280		
Vincristine	0.32					110	140
	1					115	145
	1.8					120	—

Tumor cells were inoculated ip to mice on day 0, and drugs were given ip to the mice on day 1. Median survival times in mice bearing tumor were measured. Median survival times of control group were 11.0 day in P388/MMC test, 9 day in P388/CPM test, and 10 day in P388/VCR and P388/S tests. Six mice were used in each group in all the tests. P388/MMC: Mitomycin C-resistant P388, P388/CPM: Cyclophosphamide-resistant P388, P388/VCR: Vincristine-resistant P388, P388/S: Non-resistant P388.

of new antitumor drugs[3~6]. We believe that the clinical effects of FR-900482 on a wide variety of tumors will be comparable to those of MMC, because we found that FR-900482 was active against murine leukemia, lymphoma, fibrosarcoma, melanoma, hepatoma, and mammary carcinoma, and human lung, mammary and stomach carcinomas. Against all of these tumors, FR-900482 was equally active to or more active than MMC. Regarding our results on the prolongation of the life of mice bearing MMC- or VCR-resistant tumors, INABA *et al.*[7] suggested that in anthracycline-resistant P388 cells, an active outward transport mechanism for drugs is involved in imparting resistance to the cytostatic and cytotoxic effects of drugs. TSURUO *et al.*[8] reported that verapamil, a Ca^{++} antagonist, overcame the drug resistance of tumor cells and produced renewed drug sensitivity of drug-resistant tumor cells by blocking the drug efflux process in the cells. We do not know why FR-900482 was active against MMC- and VCR-resistant P388 cells, but suppose that its mode of action is different from that of MMC or VCR. If this be the case, FR-900482 may possess a wider, or at least different antitumor spectrum from that of MMC or VCR, which are drugs of proven clinical effectiveness.

Acknowledgments

We are grateful to Ms. YOSHIE KADO for her help in preparing the manuscript.

References

1) IWAMI, M.; S. KIYOTO, H. TERANO, M. KOHSAKA, H. AOKI & H. IMANAKA: A new antitumor antibiotic, FR-900482. I. Taxonomic studies on the producing strain: A new species of the genus *Streptomyces*. J. Antibiotics 40: 589~593, 1987
2) KIYOTO, S.; T. SHIBATA, M. YAMASHITA, T. KOMORI, M. OKUHARA, H. TERANO, M. KOHSAKA, H. AOKI & H. IMANAKA: A new antitumor antibiotic, FR-900482. II. Production, isolation, characterization and biological activity. J. Antibiotics 40: 594~599, 1987
3) GOLDIN, A.; J. M. VENDITTI, J. S. MACDONALD, F. M. MUGGIA, J. E. HENNEY & V. T. DEVITA, Jr.: Current results of the screening program at the division of cancer treatment. National Cancer Institute. Eur. J. Cancer 17: 129~142, 1981
4) STAQUET, M. J.; D. P. BYAR, S. B. GREEN & M. ROZENCWEIG: Clinical predictivity of transplantable tumor systems in the selection of new drugs for solid tumors: Rationale for a three-stage strategy. Cancer Treat. Rep. 67: 753~765, 1983

5) DRISCOLL, J. S.: The preclinical new drug research program of the National Cancer Institute. Cancer Treat. Rep. 68: 63~75, 1984
6) STAQUET, M. J.; D. P. BYAR, S. B. GREEN & M. POZENCWEIG: Clinical predictivity of transplantable tumor systems in selection of new drugs for solid tumors: Reply to a commentary. Cancer Treat. Rep. 69: 1339~1340, 1985
7) INABA, M.; H. KOBAYASHI, Y. SAKURAI & R. K. JOHNSON: Active efflux of daunorubicin and adriamycin in sensitive and resistant sublines of P388 leukemia. Cancer Res. 39: 2200~2203, 1979
8) TSURUO, T.; H. IIDA, S, TSUKAGOSHI & Y. SAKURAI: Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res. 41: 1967~1972, 1981