A NEW ANTITUMOR ANTIBIOTIC, FR-900482

IV. HEMATOLOGICAL TOXICITY IN MICE

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The new antibiotic 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) possesses an antitumor activity equal to or greater than that of mitomycin C (MMC). The hematotoxicity of equivalent effective doses of the two compounds was compared in mice. A single iv injection of either compound similarly decreased the number of white blood cells (WBC) in the peripheral blood, whereas FR-900482 had no effect on the number of platelets (PTL). Both drugs slightly reduced the number of red blood cells (RBC). The effect of FR-900482 on the bone marrow cells (BMC) measured by cfu in spleen and cfu in culture was weaker than that of MMC. The results suggest that FR-900482 is a promising antitumor agent both in efficacy and safety.

FR-900482 is a new antibiotic isolated from *Streptomyces sandaensis* No. 6897 in our Research Laboratories[1]. Its chemical structure was determined to be 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[2]. We found that FR-900482 was equally effective to or more effective than mitomycin C (MMC) in experimental tumors[3]. The compound also showed antitumor activity against MMC-resistant P388 leukemia[3]. One of the main clinical side effects of chemotherapeutic antitumor drugs, especially alkylating agents and antibiotics, is myelosuppression. Thus, it was of interest to examine the hematological effects of FR-900482. This study was performed to study the acute hematotoxicity of FR-900482 and to compare this property with that of MMC, in mice.

Materials and Methods

Drugs

FR-900482 was prepared in our Research Laboratories. MMC was purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo. The drugs were dissolved in or diluted with saline just before use. The solutions were given iv at a volume of 10 ml/kg body weight. Saline was given to the control animals.

Animals

Female BDF_1 strain mice (C57BL/6×DBA/2)F_1 (BDF_1), 8 weeks old, weighing 18.0~24.0 g were used. All mice were purchased from Charles River Japan Inc., Atsugi, and maintained in Laminar Flow racks, fed CA-1 laboratory chow, and watered freely. Five mice were used per group.

Blood Cell Counts

The drugs were given iv to mice and the peripheral blood was taken on days 1, 2, 7 and 14 with a capillary from the orbital vein by gouging out the eyes. White blood cells (WBC), red blood cells (RBC) and platelets (PTL) were counted automatically with an automatic blood analyzer (Technicon Hemalog 8/90 model).

Preparation of Bone Marrow Cells

The mice were killed by cervical dislocation after collecting the peripheral blood as described above. Both ends of the femurs were cut out aseptically. The bone marrow cells (BMC) were then flushed out with a syringe with a 22-gauge needle into HANK's solution and pooled from 5 mice. A portion of the pooled bone marrow cells was stained with 0.2% trypan blue solution, and the viable nucleated cells were counted microscopically in a BURKER's hematocytometer.

Measurement of Colony Forming Units in Spleen (cfu-s)

Cfu-s were assayed according to a modification of the method of TILL and MCCULLOCH[4]. Briefly, bone marrow cells (1×10^5 cells) were given iv into recipient mice irradiated with 750 R using an X-ray irradiator (Hitachi, Irradiator HBR- 150 R) at a rate of 30 rad/minute. On day 7, the spleen of the receipient mice was removed and fixed with BOUIN's solution, and the spleen colonies were counted under a dissection microscope.

Measurement of Colony Forming Units in Culture (cfu-c)

Cfu-c were measured according to a modification of the method of PIKE and ROBINSON[5]. Briefly, 5×10^4 bone marrow cells in 1 ml of α-minimum essential medium (α-MEM) supplemented with methylcellulose 0.88%, horse serum 20% (Flow Laboratories) and penicillin and streptomycin (50 IU/ml, 50 μg/ml) were plated in a 35-mm diameter culture dishes. Twenty % of L cell- conditioned medium was used as a source of colony-stimulating factor. Triplicate cultures were made for each cell suspension, and incubated at 37°C in 5% CO_2 in humidified air. The colonies were counted 7 days later.

Results

Effects on Peripheral Blood Cells

FR-900482 and MMC were used in doses producing almost equal antitumor effects on 4 kinds of human tumor xenografts in our previous study[3].

As shown in Table 1, a single iv injection of FR-900482 decreased the number of WBC, with the maximum decrease on day 2. The WBC count returned to normal on day 7 at a dose of 5.6 mg/kg and on day 14 at doses of 10 and 18 mg/kg. The decrease in number of WBC after dosing with MMC also was greatest on day 2, and the count also returned to normal on day 7. Table 2 shows that FR-900482 in all the doses used caused a significant increase in the number of platelets on day 7, followed by a slight but significant decrease to levels below control on day 14. MMC decreased dose-dependently the number of platelets and a marked decrease was detected on day 7 in mice treated with 5.6 mg/kg of MMC. RBC decreased only slightly after dosing with either FR-900482 or MMC (data not shown).

Table 1. Effects of FR-900482 and MMC on the number of WBC in the peripheral blood.

Drug	Dose (mg/kg)	Mean±SE ($\times10^3$/mm^3)			
		Day 1	Day 2	Day 7	Day 14
Saline	0	5.0±0.53	5.1±0.44	4.5±0.11	3.9±0.35
FR-900482	5.6	3.5±0.22**	3.4±0.14**	4.2±0.56	4.8±0.57
	10	3.6±0.26*	2.2±0.13**	2.2±0.05**	3.3±0.37
	18	3.5±0.35*	2.0±0.12**	2.3±0.69*	3.3±0.31
MMC	1.8	4.0±0.36	3.9±0.15**	3.6±0.45	5.3±1.01
	3.2	2.4±0.20**	2.9±0.10**	6.1±0.71	3.7±0.21
	5.6	4.9±0.96	1.9±0.51**	5.0±0.78	3.9±0.15

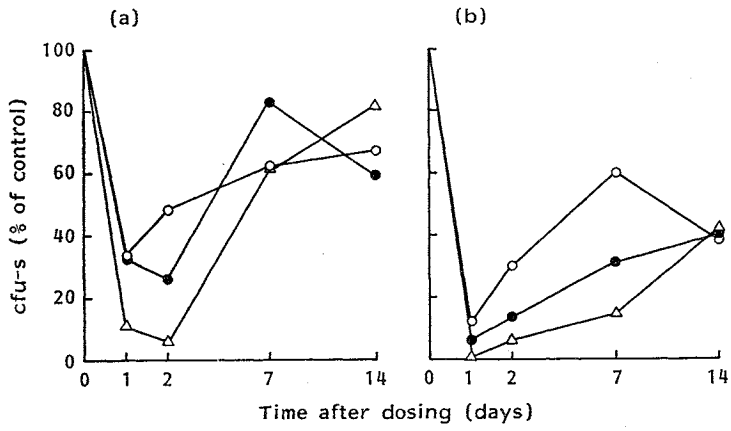
Drugs were given iv to BDF_1 mice on day 0.
Five animals were used in each treatment group on each experimental day.
* $P<0.05$. ** $P<0.01$ (comparison with saline control group).

Table 2. Effects of FR-900482 and MMC on the number of platelets in the peripheral blood.

Drug	Dose (mg/kg)	Mean±SE ($\times 10^4$/mm^3)			
		Day 1	Day 2	Day 7	Day 14
Saline	0	83±1.8	75±1.8	76±1.3	77±0.9
FR-900482	5.6	80±0.7	73±2.4	96±2.5**	73±1.0*
	10	82±1.9	71±0.7	93±2.7**	69±2.0**
	18	83±2.0	74±1.8	86±4.3	64±3.3**
MMC	1.8	83±1.2	75±1.5	74±2.8	68±0.6**
	3.2	80±0.9	78±1.7	67±2.1**	67±2.3**
	5.6	79±1.4	79±1.0	39±0.9**	60±1.8**

Drugs were given iv to BDF_1 mice on day 0.
Five animals were used in each treatment group on each experimental day.
* $P<0.05$. ** $P<0.01$ (comparison with saline control group).

Fig. 1. Effects of a single iv injection of FR-900482 and MMC on bone marrow cfu-s.
The results are expressed as percentage of control mice.
(a) FR-900482: ○ 5.6 mg/kg (iv), ● 10 mg/kg (iv), △ 18 mg/kg (iv).
(b) MMC: ○ 1.8 mg/kg (iv), ● 3.2 mg/kg (iv), △ 5.6 mg/kg (iv).



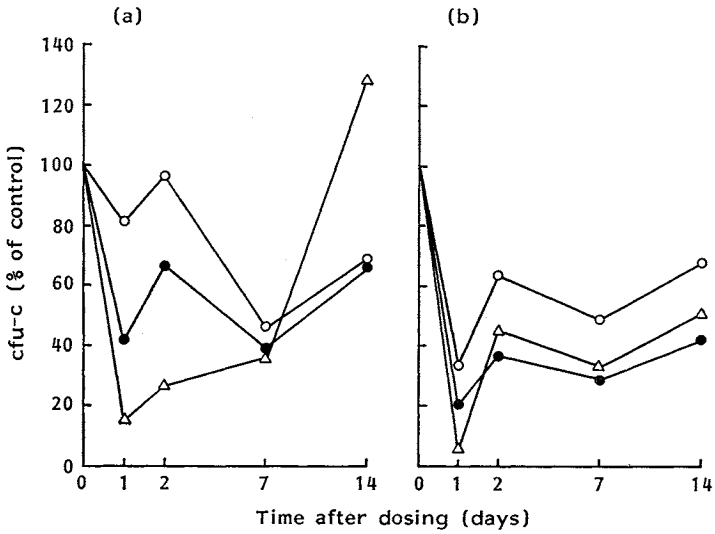
Effect on Colony Forming Units in Spleen (cfu-s)

Fig. 1 shows the time course of percent change in the cfu-s of bone marrow cells after a single iv injection of FR-900482 or MMC. The cfu-s of the FR-900482-injected mice showed a severe dose-dependent decrease on day 1 or 2, and the count gradually returned to the control level thereafter. On the other hand, the cfu-s in the MMC-injected mice showed more severe dose-dependent decreases than FR-900482 from day 1 to 14.

Effect on Colony Forming Units in Culture (cfu-c)

Fig. 2 shows the time course of the percent change in the cfu-c of bone marrow cells after a single iv dose of FR-900482 or MMC. After an injection of FR-900482 5.6 mg/kg, there was an initial decrease on day 1 with the maximum decrease occurring on day 7. Between days 7 and 14, the count gradually returned to normal. In the case of FR-900482 10 mg/kg, the maximum and equivalent decreases were observed on days 1 and 7 with a gradual recovery occurring after day 7. With FR-900482 18 mg/kg, the maximum decrease occurred on day 1, followed by a gradual recovery. MMC

Fig. 2. Effects of a single iv injection of FR-900482 and MMC on bone marrow cfu-c.
The results are expressed as percentage of control mice.
(a) FR-900482: ○ 5.6 mg/kg (iv), ● 10 mg/kg (iv), △ 18 mg/kg (iv).
(b) MMC: ○ 1.8 mg/kg (iv), ● 3.2 mg/kg (iv), △ 5.6 mg/kg (iv).



followed a similar pattern to that of FR-900482, but the decrease was somewhat greater than that observed with FR-900482.

Discussion

We used MMC for the reference compound in this hematotoxicity study because it is one of the most frequently used compounds in the treatment of cancer. FR-900482 and MMC were used in doses at which these two compounds produced almost the same degree of antitumor activity against human xenografts in our previous study[3]. A single iv injection of FR-900482 or MMC decreased similarly the number of WBC in the peripheral blood, with a maximum decrease on day 2. The effect of FR-900482 was slightly longer lasting. These two compounds exerted different effects on the number of platelets in the peripheral blood; although FR-900482 caused an increase, followed by a slight decrease, in the number of platelets, the change could be considered as within physiological range, whereas MMC significantly decreased the number of the platelets by about 50%. Both FR-900482 and MMC had only a slight effect on the number of RBC.

The effect of FR-900482 on hematopoietic stem cells and granulocytic-macrophagic precursors was studied by measuring cfu-s and cfu-c, respectively, to know its myelosuppressive effect. It is known that the marginal pool for WBC is very large and the life-span of WBC is short[5]. Thus, it is possible that merely measuring the number of WBC in the peripheral blood does not necessarily reflect the myelosuppressive effect of a drug. We showed that the effect of FR-900482 on the cfu-s and cfu-c was weaker than that of MMC. It may be necessary to study the effect of FR-900482 after repeated injections to learn more about its myelosuppressive effect.

MMC has been gaining importance in cancer chemotherapy, and it is now widely used in various combinations with other chemotherapeutics. Its main limiting factor is its hematotoxicity; hemolytic anemia[6,7], aplastic anemia[8], leukopenia[8~12], and thrombocytopenia[6,9] are all adverse effects of the drug, leukopenia and thrombocytopenia being the most frequent and serious. In the light of the excellent antitumor activity of MMC, a new compound with a comparable activity to and lesser toxicity than that of MMC has been searched for. In our previous study, FR-900482 was more effective than or equally effective to MMC[3]. In this study, we found that the leukopenic activity of FR-

900482 was slightly longer lasting than that of MMC, and that its thrombocytopenic activity and the myelosuppressive effect were weaker. Thus, there is the possibility that FR-900482 is superior to MMC both in efficacy and safety. A compound, however, with a stronger activity and less toxicity is certainly desirable. We are now looking for such a drug among the derivatives of FR-900482.

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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) SHIMOMURA, K.; O. HIRAI, T. MIZOTA, S. MATSUMOTO, J. MORI, F. SHIBAYAMA & H. KIKUCHI: A new antitumor antibiotic, FR-900482. III. Antitumor activity in transplantable experimental tumors. J. Antibiotics 40: 600~606, 1987
4) TILL, J. E. & E. A. MCCULLOCH: A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat. Res. 14: 213~222, 1961
5) PIKE, B. L. & W. A. ROBINSON: Human bone marrow colony growth in agar-gel. J. Cell. Physiol. 76: 77~84, 1970
6) GULATI, S. C.; P. SORDILLO, S. KEMPIN, L. Reich, G. B. MAGILL, E. SCHEINER & B. CLARKSON: Microangiopathic hemolytic anemia observed after treatment of epidermoid carcinoma with mitomycin C and 5-fluorouracil. Cancer 45: 2252~2257, 1980
7) PAVY, M. D.; E. L. WILEY & M. D. ABELOFF: Hemolytic-uremic syndrome associated with mitomycin therapy. Cancer Treat. Rep. 66: 457~461, 1982
8) TOMURA, T.; Y. TAKIZAWA, K. IRIKAE, O. KORENAGA, O. HIGO & A. KITAMURA: Two case of aplastic anemia with renal manifestation due to mitomycin C. Jpn. J. Cancer Clin. 12: 173~176, 1966
9) PHILIPS, F. S.; H. S. SCHWARTZ & S. S. STERNBERG: Pharmacology of mitomycin C. I. Toxicity and pathologic effects. Cancer Res. 20: 1354~1361, 1960
10) FUJIMOTO, S.: Effect of pyridoxal phosphate on toxicity and antitumor activity of mitomycin C and 4-deoxypyridoxine hydrochloride in rats — preliminary observations. Cancer Chemother. Rep. 50: 313~318, 1966
11) AKAGI, M.; S. ZAITSU, S. ANZAI, A. KIYOMOTO & K. MORI: The side effect of adjuvant cancer chemotherapy to surgery; especialy disterbance of bone marrow. Jpn. J. Cancer Clin. 12: 628~634, 1966
12) GODEREY, T. E. & D. W. WILBUR: Clinical experience with mitomycin C in large infrequent doses. Cancer 29: 1647~1652, 1972