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Antitumor Activity of BOF-A2, a New 5-Fluorouracil Derivative

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A compound containing both CNDP (3-cyano-2,6-dihydroxypyridine), an inhibitor of 5-fluorouracil (5-FU) degradation, and EM-FU (1-ethoxymethyl-5-fluorouracil), a masked form of 5-FU, was synthesized and named BOF-A2 (3-[3-(6-benzoyloxy-3-cyano-2-pyridyloxycarbonyl)benzoyl]-1-ethoxymethyl-5-fluorouracil). The antitumor activity of BOF-A2 was investigated in sarcoma-180-bearing mice and Yoshida sarcoma-bearing rats. The ED_{50} (the dose for 50% inhibition) values of BOF-A2 were 25 mg/kg against sarcoma-180 and 15 mg/kg against Yoshida sarcoma. *In vitro* studies showed that BOF-A2 was rapidly degraded to EM-FU and CNDP in homogenates of the liver and small intestine of mice and rats, and in sera of mice, rats and human, and the conversion of EM-FU to 5-FU occurred only in the microsomal fraction of rat liver in the presence of NADPH. After oral administration of BOF-A2 at 15 mg/kg to Yoshida sarcoma-bearing rats, BOF-A2 was hydrolyzed to EM-FU, CNDP and 5-FU, and their maximum concentrations in the blood were 2000 ng/ml, 300 ng/ml and 40 ng/ml, respectively. Moreover when BOF-A2 was given at the same dose to tumor-bearing mice and rats, the 5-FU levels in the tumor tissue increased much more than those in the blood and persisted for more than 8 h, whereas those in the blood decreased more rapidly. This accumulation and maintenance of a high level of 5-FU in the tumor tissue are concluded to be related to the high antitumor activity of BOF-A2.

Key words: BOF-A2 — 5-Fluorouracil derivative — Antitumor activity — Yoshida sarcoma — Sarcoma-180

5-Fluorouracil (5-FU), first synthesized in 1957,[1] has been used extensively in the treatment of certain types of cancer.[2–5] Three explanations have been given for the cytotoxic action of 5-FU: one widely accepted explanation is that 5-fluorodeoxyuridine 5′-monophosphate (EdUMP), an active metabolite of 5-FU, inhibits thymidylate synthase (EC 2.1.1.45) irreversibly.[6–11] Another explanation is that 5-FU is incorporated into RNA,[12–15] and distorts gene expression.[12, 16] A third explanation is that 5-FU is incorporated into DNA and interferes with DNA metabolism.[17–19]

However, 5-FU is rapidly degraded by dihydrouracil dehydrogenase (EC 1.3.1.2), mainly in the liver, and is excreted in the urine as 2-fluoro-β-alanine.[20–22]

In 1978, we reported that uracil potentiated the antitumor activity of 5-FU and tegafur, which was found as an antitumor agent by Hiller *et al.*,[23] and is mainly converted to 5-FU in the microsomal fraction of the liver,[24] and in sarcoma-180 in mice and AH-130 hepatoma in rats,[25, 26] and we concluded that this potentiation was due to inhibition of the degradation of 5-FU, without affecting the phosphorylation of 5-FU.[20]

In studies on potentiation of the antineoplastic activity of 5-FU, Desgranges *et al.* found in 1986 that (*E*)-5-(2-bromovinyl)uracil caused irreversible inhibition of the reductive step in pyrimidine degradation catalyzed by dihydrothymine dehydrogenase, and increased the mean survival time of P388-bearing mice about 1.4-fold over that of animals treated with 5-FU only.[27]

In further investigations on the metabolism of fluorinated pyrimidine, we recently found potent inhibitors of the degradation of 5-FU such as 3-cyano-2,6-dihydroxypyridine (CNDP), which is about 300-fold more inhibitory than uracil *in vitro*.[28] Moreover, we also found that 1-ethoxymethyl-5-fluorouracil (EM-FU) was a masked form of 5-FU.[29] Therefore, a compound containing both CNDP and EM-FU was synthesized with the aim of potentiating the selective cytotoxicity of 5-FU in our institute[30] and named BOF-A2.

The present paper reports the antitumor activity of BOF-A2 in sarcoma-180-bearing mice and Yoshida sarcoma-bearing rats, and discusses the reason for its effectiveness.

MATERIALS AND METHODS

Chemicals 5-FU and nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) were purchased from Sigma Chemical Co., MO. All other chemicals were commercial products. BOF-A2(3-[3-(6-benzoyloxy-3-cyano-2-pyridyloxycarbonyl)benzoyl]-1-ethoxymethyl-5-fluorouracil), CNDP and EM-FU were synthesized in this institute.

Animals Donryu and Wistar-King strain rats weighing 100–120 g and ICR mice weighing 20–25 g were purchased from Laboric Service Co., Shiga. They were supplied *ad libitum* with commercial diet and autoclaved water until used in experiments.

Tumor cells Sarcoma-180 and Yoshida sarcoma cells were supplied by Sasaki Research Institute, Tokyo, and passaged in male ICR mice and male Donryu strain rats, respectively, by intraperitoneal inoculation at weekly intervals.

Chemotherapy Groups of 8 mice were used. Solid-type sarcoma-180 was prepared by implanting 2×10^7 tumor cells into subepidermal tissues of the back of mice on day 0. BOF-A2, suspended in 1% hydroxypropylmethyl cellulose solution, was administered orally once daily for 7 consecutive days, starting 24 after implantation of tumor cells. Control mice were given 1% hydroxypropylmethyl cellulose solution alone by the same schedule. On day 10, the mice were killed, and the tumors were removed and weighed. The antitumor activity of the drugs was evaluated according to the following formula.

$$\text{T/C (\%)} = \frac{\text{mean tumor weight in drug-treated mice}}{\text{mean tumor weight in control mice}} \times 100$$

Groups of 7 rats were used. Solid-type Yoshida sarcoma was prepared by transplantation of 1×10^4 cells subepidermally into the back of Donryu rats and the experiments were carried out as in mice, except that the rats were sacrificed on day 8. 5-FU, dissolved in saline, was injected intraperitoneally on the same schedule.

Extraction and determination of 5-FU from blood, tumors and other tissues of sarcoma-180-bearing mice and Yoshida sarcoma-bearing rats Drugs were administered orally to sarcoma-180-bearing mice on day 10 after tumor implantation, or to Yoshida sarcoma-bearing rats on day 8 after tumor implantation. Then the mice and rats were sacrificed at various times and their blood, tumors and other tissues were rapidly removed. The tissues were homogenized with 3 volumes of ice-cold acetonitrile, the homogenates were centrifuged at 3,000 rpm for 10 min, and the resultant supernatants were dried and used as crude extracts of 5-FU. The blood samples or crude extracts from tissues was shaken with 5 ml of chloroform for 10 min. The mixtures were centrifuged at 3,000 rpm, and the organic layer (containing EM-FU) was removed. 5-FU in the remaining aqueous layer was extracted with 4 ml of ethyl acetate, twice, and the two ethyl acetate layers were combined and evaporated at 40°C under a stream of nitrogen gas. The residues were dissolved in distilled water, and passed through a 0.45 μm filter, and the 5-FU contents of the filtrates were determined by reverse-phase high-performance liquid chromatography (HPLC). 5-FU was measured in a BIP-I HPLC system (JASCO, Tokyo). For this, aliquots of the sample were applied to a column (4.6 ID$\times$150 mm for blood, 4.6 ID$\times$250 mm for tumor extracts) of ULTRON N-C_{18}·L (Shinwa-Kako Co. Ltd, Kyoto), under the following chromatographic conditions: monitoring wavelength, 280 nm; flow rate, 1 ml/min; mobile phase, 5 m*M* tetrabutylammonium solution containing 2% methanol adjusted to about pH 5 with dilute formic acid.

Enzymatic hydrolysis of BOF-A2 *in vitro* All procedures for preparation of enzyme solutions were carried out at 4°C. Tissue samples (1–2 g weight) were minced with scissors and homogenized with 4 volumes of 50 m*M* Tris-HCl buffer (pH 8.0), containing 5 m*M* 2-mercaptoethanol. The homogenates were centrifuged (9,000*g*, 20 min) and the supernatants were used for assays of hydrolysis of BOF-A2. Serum samples from mice, rats and humans were also used as enzyme sources. The reaction mixture, in a total volume of 0.5 ml, consisted of 1 m*M* BOF-A2, 50 m*M* Tris-HCl buffer (pH 8.0) and enzyme solution (0.4 ml). After incubation at 37°C, the reaction was terminated by addition of ice-cold methanol (2 ml), and the mixture was centrifuged (3000 rpm, 10 min). EM-FU and CNDP produced from BOF-A2 were measured in the same chromatographic apparatus. For EM-FU, aliquots of the sample were applied to a column (4.6 ID $\times$ 150 mm) of CHEMCOSORB 300-5 C_{18} (Chemco Co., Ltd., Osaka), under the following chromatographic conditions: monitoring wavelength, 280 nm; flow rate, 1 ml/min; mobile phase. 10% CH_3CN containing 0.01% tetrafluoroacetate. For CNDP, aliquots of the sample were applied to the same column under the following chromatographic conditions: monitoring wavelengths, Ex 330 nm and Em 380 nm (fluorescence); flow rate, 1 ml/min; mobile phase, 4% CH_3CN containing 0.01% tetrafluoroacetate. The values (%) of EM-FU and CNDP produced was calculated from the results of analysis of a standard reaction mixture containing 1 m*M* EM-FU and 1 m*M* CNDP instead of BOF-A2.

Conversions of EM-FU and tegafur to 5-FU by the microsomal fraction of rat liver Preparation of liver microsomes and assay of cytochrome P-450 activity were carried out as described by Au and Sadee.[31] Male Wistar-King rats (7 weeks old) were decapitated, and their livers were removed and homogenized with 0.25 *M* sucrose containing 5m*M* 2-mercaptoethanol. The homogenates were centrifuged at 9,000*g* for 30 min at 4°C and the supernatant at 105,000*g* for 60 min. The 105,000*g* microsomal pellet was suspended in 10 m*M* potassium phosphate buffer containing 1.15% KCl (pH 7.4) and used as an enzyme source. The reaction mixture consisted of 1 ml of microsome suspension (1 g liver/ml), 0.5 ml of 0.45 m*M* $MgCl_2$, 0.25 ml of 20 m*M* NADPH and 0.1 ml of 10 m*M* EM-FU or tegafur. The mixture was incubated with vigorous shaking at 37°C. After 30 and 60 min, further NADPH solution was added and incubation was continued. After the indicated periods of incubation, 0.1 ml of 1 *N* HCl and 8 ml of ethyl acetate were added and 5-FU was extracted by shaking with ethyl acetate for

10 min. Subsequent treatment of the sample and determination of 5-FU were as described above. The values (%) of 5-FU produced was calculated from the analysis of a standard mixture containing 5-FU instead of EM-FU or tegafur.

RESULTS

Effects of an inhibitor (CNDP) of 5-FU degradation on antitumor activity of EM-FU in sarcoma-180-bearing mice Sarcoma-180-bearing mice were treated orally with

Table I. Effect of CNDP on Antitumor Activity of EM-FU in Sarcoma-180-bearing Mice

Drugs	Dose (mg/kg)	Tumor weight (g±SE)	T/C (%)	Body weight changes (%)
Control	—	1.11±0.12	100.0	100.0
EM-FU alone	2	0.92±0.17	82.9	125.4
	5	0.86±0.12	77.4	105.8
	10	0.89±0.15	80.1	93.5
	20	0.94±0.10	84.7	119.4
EM-FU plus CNDP (mol. ratio 1:1)	2	0.69±0.10	62.2	110.6
	5	0.47±0.06	42.3	113.8
	10	0.24±0.03	21.6	14.3
	20	0.00±0.00	0	−51.5
CNDP alone	4	1.07±0.24	96.4	95.0
	8	1.16±0.22	104.5	103.0
	16	1.21±0.29	109.0	80.2

EM-FU, CNDP and EM-FU plus CNDP were orally administered to sarcoma-180-bearing mice once daily for 7 consecutive days, starting 24 h after the tumor implantation. On day 10, mice were sacrificed and their tumors were weighed. The antitumor effects of the drugs were evaluated in 8 mice per group.

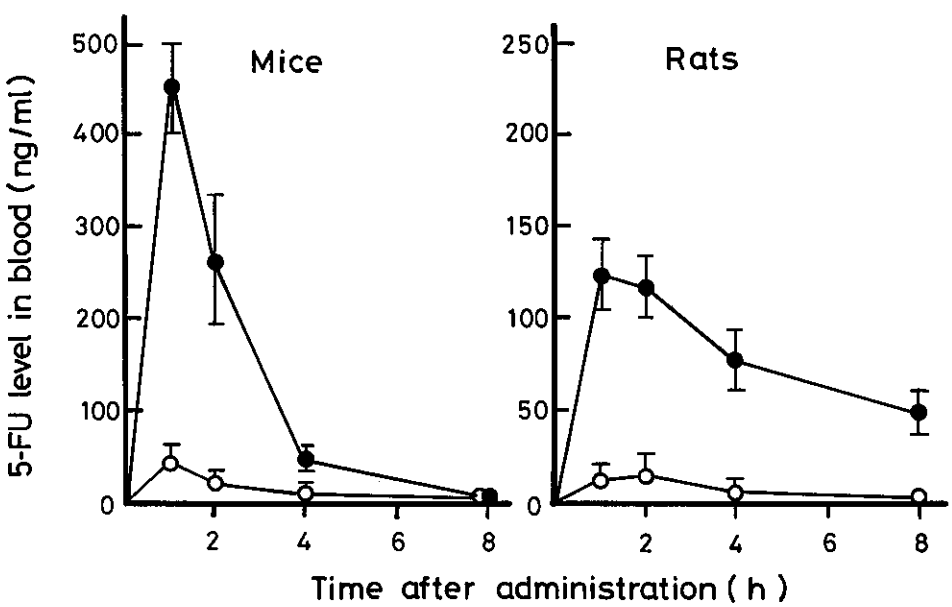

Fig. 1. Effect of oral co-administration of CNDP and EM-FU on the 5-FU levels in the blood of tumor-bearing mice and rats. EM-FU (5 mg/kg) combined with equimolar CNDP or without CNDP was administered orally to sarcoma-180-bearing mice and Yoshida sarcoma-bearing rats. 1, 2, 4 and 8 h later, 5-FU levels in the blood were determined. EM-FU alone, ○; EM-FU plus CNDP, ●; values are means±SE for 3 mice and rats.

EM-FU (1-ethoxymethyl-5-fluorouracil) plus an equimolar amount of CNDP (3-cyano-2,6-dihydroxypyridine) and the antitumor activities and toxicities of the drugs were measured. The results are shown in Table I. A significant inhibition of the tumor growth was effected by oral EM-FU in combination with CNDP. About 50% inhibition of tumor growth was attained at the dose of 5 mg/kg, and the mice given of EM-FU plus CNDP at the dose of 20 mg/kg were tumor-free, though EM-FU alone

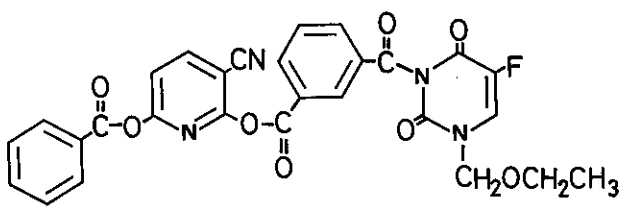

Fig. 2. Chemical structure of BOF-A2.

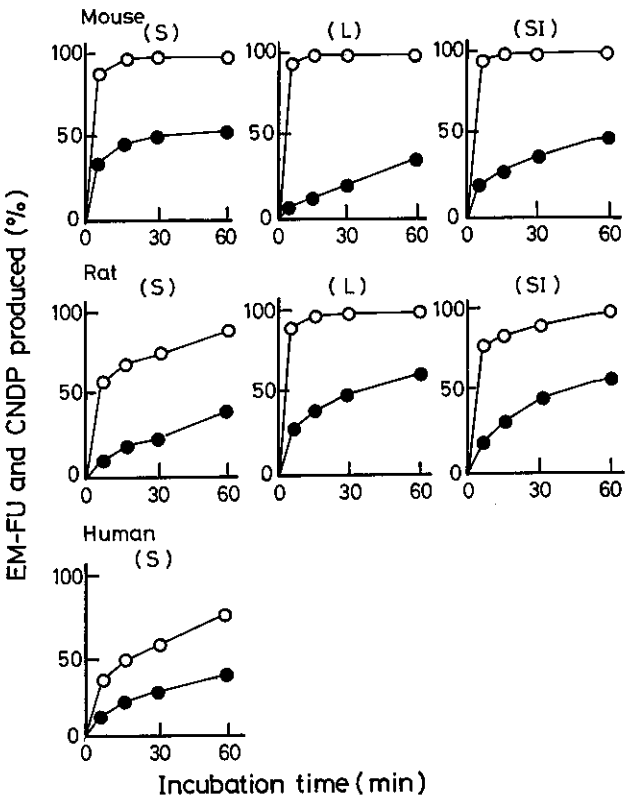

Fig. 3. Enzymic conversion of BOF A2 to EM-FU and CNDP in serum, liver and small intestine of mice and rats, and human serum *in vitro*. BOF-A2 (1 m*M*/assay mixture) was incubated with enzyme solution obtained from the liver or small intestine of mice and rats, or with mouse, rat or human serum. After 5, 15, 30 and 60 min, the reaction was terminated and the products, EM-FU (●) and CNDP (○), were measured as described in "Materials and Methods." Abbreviations used are: (S), serum; (L), liver; (SI), small intestine.

Table II. Production of 5-FU from EM-FU and Tegafur in Preparations from Various Rat Tissues *in vitro*

Enzyme source	Tissue	5-FU formed (%)	
		from EM-FU	from tegafur
50 m*M* Tris-HCl (pH 8.0)		0	0.16
33% homogenate 9,000*g*, sup.	Liver	0	0.03
	Kidney	0	0
	Spleen	0	0
	Small intestine	0	0.01
	Lung	0	0
	Heart	0	0
	Thymus	0	0
	Serum	0	0
Microsomes (105,000*g*, pellet)	Liver	0.46	0.92

EM-FU, and tegafur as a positive control (1 m*M* each), were incubated with the indicated tissue homogenates of rats or the microsome fraction of rat liver in the presence of NADPH. After 60 min, the reaction was terminated and the amount of 5-FU produced determined as described in "Materials and Methods."

Table III. Antitumor Effects of BOF-A2 and 5-FU in Sarcoma-180-bearing Mice and Yoshida Sarcoma-bearing Rats

Drugs	Dose (mg/kg)	n	Tumor wt. (g±SE)	T/C (%)	Body wt. change (%)
Sarcoma-180					
Control	—	16	0.812±0.095	100.0	100.0
BOF-A2	5	8	0.710±0.145	87.5	96.5
(MW:558.48)	10	8	0.530±0.080	65.2	89.6
	20	8	0.477±0.066	58.7	81.4
	40	8	0.356±0.036	43.8	41.7
	60	5[a]	0.161±0.029	19.8	−34.1
	80	1[a]	0.103	12.7	−38.1
Control	—	16	0.640±0.103	100.0	100.0
5-FU	10	8	0.641±0.103	100.0	91.4
(MW:130)	20	8	0.504±0.057	78.7	67.9
	30	4[a]	0.290±0.035	45.3	8.5
	40	3[a]	0.124±0.028	19.4	−51.7
Yoshida sarcoma					
Control	—	16	1.74±0.11	100.0	100.0
BOF-A2	5	7	1.59±0.10	91.4	98.4
(MW: 558.48)	10	7	1.10±0.15	63.2	63.1
	20	7	0.37±0.16	21.3	15.2
	40	5[b]	0.00±0.00	0	−35.0
Control	—	7	1.17±0.10	100.0	100.0
5-FU	5	7	1.42±0.18	121.4	114.4
(MW: 130)	10	7	0.91±0.12	77.8	78.9
	20	7	0.49±0.13	41.9	42.4
	40	2[b]	0.00±0.00	0	−110.3

BOF-A2 was administered orally and 5-FU was injected intraperitoneally into sarcoma-180-bearing mice and Yoshida sarcoma-bearing rats. The antitumor effects of the drugs were evaluated as described in "Materials and Methods."

a) Number of surviving animals on day 10.

b) Number of surviving animals on day 8.

had almost no antitumor activity, even at 20 mg/kg. Scarcely any change in body weight was observed with the combination of EM-FU and CNDP at about the ED_{50} (the dose for 50% inhibition) value.

5-FU levels in the plasma of mice and rats after oral co-administration of EM-FU and CNDP To elucidate the mechanism of potentiation of the antitumor activity mentioned above, mice and rats were given an oral dose of EM-FU (5 mg/kg) alone or EM-FU (5 mg/kg) plus an equimolar amount of CNDP (4.1 mg/kg), and 1, 2, 4 and 8 h after drug administration, the 5-FU levels in the blood were determined. As shown in Fig. 1, high 5-FU concentrations were found in the blood of mice and rats after administration of EM-FU plus CNDP. After treatment with EM-FU alone, 5-FU levels in blood were very low. These results suggest that enhancement of the antitumor activity of EM-FU was obtained by treatment with CNDP, which is a potent inhibitor of the degradation of 5-FU.

***In vitro* studies of BOF-A2** A new chemical compound, 3-[3-(6-benzoyloxy-3-cyano-2-pyridyloxycarbonyl)-benzoyl]-1-enthoxymethyl-5-fluorouracil, was synthesized from CNDP and EM-FU, the masked form of 5-FU, and named BOF-A2 (Fig. 2). The enzymic conversion of BOF-A2 to EM-FU and CNDP in mouse and rat tissues and human serum was examined. As shown in Fig. 3, with serum and homogenates of liver and small intestine of rodents, BOF-A2 was rapidly degraded by esterase or N-acylase and the resulting level of CNDP was much higher than that of EM-FU; similar degradation was observed with human serum. As shown in Table II, in *in vitro* studies with 9,000*g* supernatants of liver, kidney, spleen, small intestine, lung, heart, thymus, serum and buffer, no formation of 5-FU from EM-FU was detected

during incubation for 60 min at 37°C. We found that EM-FU was converted to 5-FU only by the microsomal fraction of the liver in the presence of NADPH. This finding suggested that the microsomal electron transport system was involved in the conversion of EM-FU to 5-FU, as in the case of tegafur.

Antitumor activity of BOF-A2 against sarcoma-180 in mice and Yoshida sarcoma in rats Sarcoma-180-bearing mice and Yoshida sarcoma-bearing rats were treated with BOF-A2 or 5-FU and the T/C values were calculated. The results are shown in Table III. Significant reduction in tumor weight was found in mice treated with BOF-A2. The dose for 50% inhibition (T/C 50) of tumor growth was about 30 mg/kg. In Yoshida sarcoma-bearing rats, BOF-A2 was even more effective, and about 50% inhibition of tumor growth was attained at a dose of 15 mg/kg of BOF-A2. The T/C values on oral administration of BOF-A2 were similar to those of intraperitoneal injection of 5-FU. Although there is no statistically significant difference between the ED_{50} values (mg/kg) of BOF-A2 and 5-FU, the antitumor activity of BOF-A2 on an equimolar basis (μmol/kg) is higher than that of 5-FU, because the molecular weight of BOF-A2 (MW 558.5) is about 4 times higher than that of 5-FU (MW 130.1).

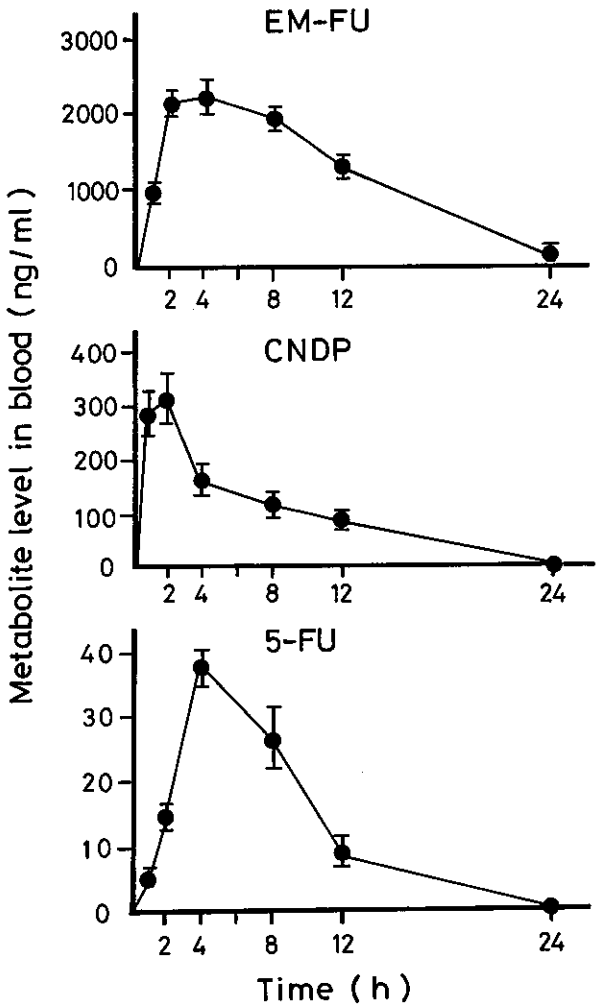

Fig. 4. Levels of EM-FU, CNDP and 5-FU in the blood of Yoshida sarcoma-bearing rats after oral administration of BOF-A2. Yoshida sarcoma-bearing rats were treated orally with BOF-A2 at 15 mg/kg suspended in 1% hydroxypropylmethyl cellulose solution on day 8 after tumor implantation. After the indicated periods, whole blood samples (5 ml) were removed and the serum was obtained by centrifugation (3000 rpm, 10 min). The metabolites EM-FU, CNDP and 5-FU were extracted from the serum and measured as described in "Materials and Methods." Values are means±SE for 5 rats.

Concentrations of EM-FU, CNDP, and 5-FU in the blood of Yoshida sarcoma-bearing rats after oral administration of BOF-A2 On day 8 after tumor implantation, Yoshida sarcoma-bearing rats were given BOF-A2 (15 mg/kg) orally, and 1, 2, 4, 6, 8, 12, 24 and 48 h later, the concentrations of EM-FU, CNDP and 5-FU in their blood were determined. As shown in Fig. 4, the EM-FU level in the blood was more than 2,000 ng/ml for the first 4 h and remained at more than 1,000 ng/ml for at least 12 h, while the CNDP level was more than 300 ng/ml for the first 2 h and remained at more than 100 ng/ml for at least 12 h, and the 5-FU level in the blood increased to a maximum 4 h after BOF-A2 administration and remained at more than 10 ng/ml for at least 12 h. Thus the concentrations of EM-FU, CNDP and 5-FU in the blood persisted at high levels for at least 12 h after administration of BOF-A2.

5-FU levels in the blood and tumor tissues of sarcoma-180-bearing mice after administration of BOF-A2 On day 10 after implantation of sarcoma-180, mice were given BOF-A2 (15 mg/kg) orally, and 1, 2, 4, 8, 12 and 24 h later, the 5-FU levels in their tumor tissue and blood were determined. As shown in Fig. 5, the 5-FU levels in the blood of mice were more than 200 ng/ml for the first 1 h and then decreased rapidly, whereas the 5-FU levels in the tumor tissue remained at more than 200 ng/ml for 2 h. After administration of BOF-A2, high 5-FU concentrations were found in the tumor tissue after 4 to 8 h. Since the toxicity of 5-FU seems to be proportional to its concentration in the blood, it is important to keep its level low in the blood, but high in tumor tissue.

5-FU levels in the tumor and various organs of Yoshida sarcoma-bearing rats Yoshida sarcoma-bearing rats on day 8 after implantation were given BOF-A2 (15 mg/kg) by oral administration. Then 2, 4, 8, 12 and 24 h after drug administration, the tumors and various organs were removed, and their 5-FU levels were determined. As shown in Table IV, the values of the ratio of 5-FU level

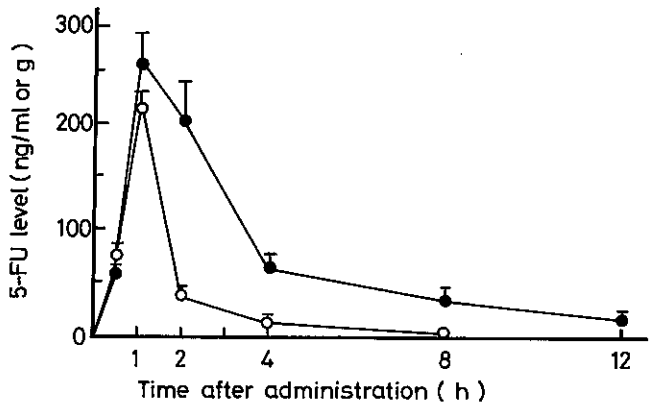

Fig. 5. 5-FU levels in the blood and tumor tissue of sarcoma-180-bearing mice after oral administration of BOF-A2. BOF-A2 at 15 mg/kg, suspended in 1% hydroxypropylmethyl cellulose solution, was given orally to sarcoma-180-bearing mice on day 10 after tumor implantation. Mice were killed and their blood and tumors were promptly removed and stored at −20°C until use. 5-FU levels in the blood (○) and tumor (●) were determined as described in "Materials and Methods." Values are means±SE for 5 mice.

Table IV. 5-FU Levels in the Blood, Tumor and Normal Tissues of Yoshida Sarcoma-bearing Rats after Oral Administration of BOF-A2

Time (h)	5-FU levels (ng/ml, or ng/g)							
	Serum	Tumor	Liver	Kidney	Spleen	Small intestine	Lung	Heart
2	15±1	54±8	ND	52±20	48±12	33±5	20±2	3±2
4	38±3	101±4	5±2	125±10	46±8	27±6	34±4	36±6
8	26±5	170±40	6±2	118±11	73±16	13±4	54±7	10±5
12	9±3	93±14	10±2	26±6	61±16	4±4	43±11	ND
24	ND	50±14	ND	11±6	8±1	ND	7±4	ND

BOF-A2 at 15 mg/kg was administered orally, and 2, 4, 8, 12 and 24 h later, the rats were killed and 5-FU levels in various tissues were determined as described in "Materials and Methods." Values are means ±SE for 4 to 5 rats. ND: not detectable.

in the tumor to that in the blood were 3.6, 2.7, 6.5 and 10.3 at 2, 4, 8 and 12 h, respectively. There was a significant difference between the blood and tumor, and the 5-FU level in the blood of rats given BOF-A2 decreased more rapidly than that in the tumor. Thus the ratio of 5-FU level in the tumor to that in the blood increased with time after drug administration. It is noteworthy that after administration of BOF-A2, the difference between the 5-FU levels in the blood and tumor tissue was greater in rats than in mice.

These results suggest that BOF-A2 was more effective in rats than mice because it yielded high 5-FU levels in the tumor tissue for a long time and because the difference between the 5-FU levels in the tumor tissue and blood was greater.

DISCUSSION

Previously, we found that the antitumor activity of tegafur against sarcoma-180 and AH-130 tumors was enhanced by simultaneous oral administration of uracil, thymine, uridine, thymidine or cytosine, and that the 5-FU concentration in tumors after administration of tegafur plus uracil was much higher than that after administration to tegafur alone.[25,26,32] This enhancement of the antitumor activity by uracil was due to its inhibition of 5-FU degradation, which is catalyzed by dihydrouracil dehydrogenase.

Recently, we found that CNDP was a potent inhibitor of dihydrouracil dehydrogenase with a K_i value of 3.6×10^{-7} *M*.[28] This inhibitor is 300-fold more inhibitory than uracil, and inhibited 5-FU degradation without influencing its phosphorylation appreciably. Desgranges *et al.*[27] reported that in DBA/2 mice inoculated with 10^6 P388 leukemia cells, pretreatment with (E)-5-(2-bromovinyl)-uracil (BVUra) (IC_{50}: $2–3\times10^{-6}$ *M*) enhanced the antitumor activity of 5-FU, and that BVUra increased the plasma half-life of 5-FU in DBA/2 mice by decreasing 5-FU degradation.

These findings led us to investigate the antitumor effects of combinations of EM-FU and CNDP. In this study, the antitumor activities of EM-FU in combination

with CNDP were stimulated markedly in sarcoma-180-bearing mice. When EM-FU in combination with CNDP was administered to tumor-bearing mice and rats, the 5-FU levels in the blood increased much more than after EM-FU alone.

These results prompted us to synthesize a compound containing the masked form of 5-FU, EM-FU, and the inhibitor of 5-FU degradation, CNDP. The resulting compound was named BOF-A2 (3-[3-(6-benzoyloxy-3-cyano-2-pyridyloxycarbonyl)benzoyl]-1-ethoxymethyl-5-fluorouracil). Referring to our previous reports,[25,26] the antitumor activity of BOF-A2 is even more potent than that of UFT against sarcoma-180 tumor in mice.

Various inhibitors enhance both the antitumor activity and the toxic effects of 5-FU, probably by inhibiting 5-FU degradation, but a masked form of 5-FU such as EM-FU, which is slowly converted to 5-FU only by the microsomal fraction, seems to produce a suitable balance between antitumor activity and toxic effects.

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