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Oral Administration of BOF-A2 to Rats with Lung Transplanted Tumors Results in Increased 5-Fluorouracil Levels

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A new tumor model was developed in which solid Yoshida sarcoma tissue injected intravenously developed into tumors in the lungs of about 30% (13/42) of the inoculated rats. Histological examination revealed that alveoli were occupied with tumor cells and the tumors were similar to those obtained by subcutaneous inoculation. Using this model, the concentrations of 5-fluorouracil (5-FU) in tumor tissue 12 h after oral administration of 3-[3-(6-benzoyloxy-3-cyano-2-pyridyloxycarbonyl)-benzoyl]-1-ethoxymethyl-5-fluorouracil (BOF-A2, 36 μmol/kg) or a combination of tegafur and uracil in a molar ratio 1 to 4 (UFT, 50 μmol/kg) were found to be 129.8 ng/g and 19.2 ng/g, respectively. Thus, compared to treatment with UFT, BOF-A2 resulted in higher levels of 5-FU in lung tumor tissues. Increased concentrations of 5-FU should have a superior anti-tumor effect and so BOF-A2 merits clinical trial in the treatment of patients with lung cancer.

Key words: BOF-A2 — 5-Fluorouracil — Lung tumor tissue — Lung transplanted tumor model

Many patients with breast, gastric and colo-rectal cancers have been treated with fluorinated pyrimidines such as 5-fluorouracil (5-FU), tegafur, and carmofur. However, because of the rapid degradation of 5-FU, the active form of these compounds, to F-β-alanine by dihydrouracil (DHU) dehydrogenase,[1-4] these agents have not been as effective as expected.

Fujii *et al.*[5,6] discovered that the addition of uracil, a competitive inhibitor of 5-FU catabolism, caused an elevation of 5-FU concentrations in tumor tissues. They developed UFT, composed of 1-(2-tetrahydrofuryl)-5-fluorouracil (tegafur) and uracil in the molar ratio of 1 to 4. Other investigators have also reported a possible correlation of higher 5-FU concentration with superior anti-tumor effect.[7-11]

Hirohashi *et al.*[12] recently synthesized BOF-A2, which is composed of 1-ethoxymethyl-5-fluorouracil (EM-FU) and 3-cyano-2,6-dihydroxypyridine (CNDP), a 2000-fold more potent inhibitor of 5-FU degradation than uracil.[13] BOF-A2 has been shown to have potent anti-tumor effects on various experimental tumors[14] and human lung cancers xenografted into nude mice.[15]

In this study, we examined 5-FU levels in tumor tissues after BOF-A2 or UFT administration by using a rat model with transplanted tumor in the lung to investigate whether or not BOF-A2 would give higher 5-FU levels in the tumor.

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MATERIALS AND METHODS

Chemicals 3-[3-(6-Benzoyloxy-3-cyano-2-pyridyloxycarbonyl)benzoyl]-1-ethoxymethyl-5-fluorouracil (BOF-A2), EM-FU and CNDP were synthesized at Otsuka Pharmaceutical Co. 5-FU was purchased from Daikin Industrial Co. Tegafur was purchased from Asahi Kasei Industrial Co. UFT was purchased from Taiho Pharmaceutical Co. Hydroxypropyl methylcellulose (Lot No. 910308) was purchased from Shin-Etsu Chemical Co. All other chemicals were standard commercial products. Chemical structures of BOF-A2 and UFT are shown in Fig. 1.

Preparation of rats with Yoshida sarcomas in the lung Yoshida sarcoma cells were passaged in 5- to 6-week-old male Donryu rats by intraperitoneal inoculation at weekly intervals. Solid-type Yoshida sarcoma tumors were prepared by implanting 5×10^4 tumor cells into subcutaneous tissues in the back of rats. After 10 days, the tumors were removed under sterile conditions and cut into blocks of about 0.5 mm×0.5 mm. A block of solid tumor was then injected intravenously into the left jugular vein of 42 rats for histological examination and into 290 rats for determination of 5-FU, EM-FU and CNDP level, using a 19G needle. Ten days after intravenous tumor injection, 42 rats were killed and their lungs were removed to determine the incidence of tumors and for histological examination of the lung tumors, in order to compare them with subcutaneously inoculated tumors.

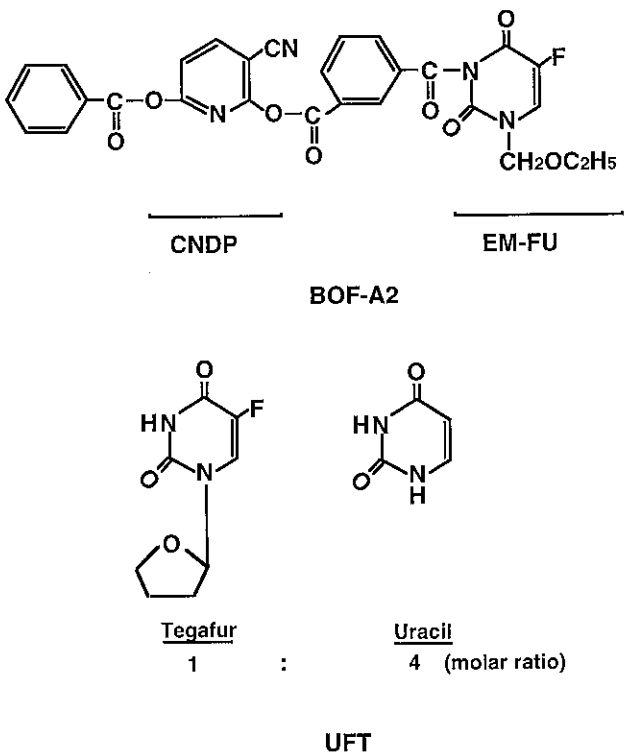

Fig. 1. Chemical structures of BOF-A2 and UFT.

Histological examination Tumor tissues were fixed in 10% buffered formaldehyde solution and sections were routinely stained with hematoxylin and eosin.

Preparation of plasma, lung and tumor tissue samples after oral administration of BOF-A2 or UFT We employed dosages of 36 μmol/kg BOF-A2 and 50 μmol/kg UFT to compare the tumor 5-FU content after administration of the two agents. Each agent, suspended in 1% hydroxypropyl methylcellulose, was given orally to 145 rats. Ten days after tumor inoculation, rats were killed at 2, 6 or 12 h after agent administration and 5 rats in each group with large tumors (0.5–2 g), easily distinguished from normal lung tissue, were used for determination of drug concentrations in their plasma and normal lung or tumor tissues. Each sample was stored at -80°C until determinations of metabolites (5-FU, EM-FU and CNDP) from BOF-A2-treated rats or 5-FU from those given UFT.

Determinations of EM-FU, CNDP and 5-FU concentrations in tissues and plasma The tissues were homogenized in 3 volumes of acetonitrile and centrifuged at 3,000 rpm for 10 min. The supernatants were dried under an air stream and the residues were dissolved in 1 ml of 0.05 *M* borate buffer (pH 10) and shaken with 3 ml of ethyl acetate for 10 min. The mixtures were centrifuged at 3,000 rpm for 10 min, and the organic layer was removed. The aqueous layer was shaken with 50 μl of 1 *M* hydrochloric acid, 1 ml of saturated ammonium sulfate and 5 ml of chloroform for 10 min and centrifuged at 3,000 rpm for 10 min. The organic layer was evaporated to dryness at 40°C under an air stream and the residue was dissolved in 30% methanol as the EM-FU sample. The aqueous layer was shaken with 3 ml of ethyl acetate twice, and the two ethyl acetate layers were combined and evaporated to dryness at 40°C under an air stream. The residue was dissolved in distilled water as the 5-FU sample.

Samples of plasma were mixed with 1 ml of saturated ammonium sulfate and shaken with 5 ml of chloroform for 10 min. The organic layer, separated by centrifugation at 3,000 rpm for 10 min, was evaporated to dryness at 40°C under an air stream and the residue was dissolved in 30% methanol as the EM-FU sample. The aqueous layer was shaken with 3 ml of ethyl acetate twice, and the two ethyl acetate layers were combined and evaporated to dryness at 40°C under an air stream. The residue was dissolved in distilled water as the 5-FU sample.

CNDP was determined by another extraction method. The plasma preparations were mixed with 0.4 ml of acetonitrile in a vortex mixer and centrifuged at 3,000 rpm for 10 min. The supernatants were evaporated to dryness under an air stream at below 40°C, and the residues were dissolved in 100 μl of HPLC elution buffer as CNDP samples.

Aliquots of samples were applied to a reverse-phase ODS column (YMC A-302, 4.6 mmID $\times$ 150 mm for EM-FU and CNDP; YMC A-303, 4.6 mmID $\times$ 250 mm for 5-FU) under the following chromatographic conditions: monitoring wavelength; 280 nm, flow rate; 1 ml/min. The mobile phases were as follows: acetonitrile/acetic acid/water (10/1/89) for EM-FU, acetonitrile/0.1 *M* acetate buffer pH 5.0 (1/99) for CNDP, 0.1% acetic acid for 5-FU in plasma samples, and 1 m*M* acetate buffer (pH 6.0) for 5-FU in tissue samples. Columns were purchased from YMC Co., Kyoto.

Statistical analysis Results are given as mean $\pm$ SD. The significance of differences of values between plasma, normal lung tissue and lung tumor tissue in the same agent group was analyzed by use of the Tukey test, and that of differences of values in tumors between the two agents was evaluated by using the Wilcoxon rank sum test.

RESULTS

Histological findings Lung tissue was removed 10 days after intravenous injection of a block of tumor tissue. Solid tumors were found in the lung and their incidence in the lung was approximately 30% (13/42, 10 in the right and 3 in the left lung). The alveoli were occupied

Table I. 5-FU Concentration in Plasma, Normal Lung and Tumor Tissues after Oral Administration of BOF-A2 (36 μmol/kg) or UFT (50 μmol/kg) to Rats with Lung Transplanted Tumors[a)]

	Plasma (ng/ml)		Normal lung (ng/g)		Tumor (ng/g)	
	UFT	BOF-A2	UFT	BOF-A2	UFT	BOF-A2
2 h	12.0±1.6	24.0±7.3	59.6±99.7	30.0±10.2	80.2±101.1 (6.7)[b)]	49.8±27.7[d)] (2.1)[b)] (0.6)[c)]
6 h	6.6±3.9	32.6±19.2	63.4±16.7	89.6±41.9	90.4±42.4[d)] (13.7)	216.8±117.7[d)] (6.7) (2.4)
12 h	3.6±5.4	23.2±7.3	24.4±18.2	68.0±25.9	19.2±11.3 (5.3)	129.8±53.0[e,f)] (5.6) (6.8)

a) Results are shown as mean±SD from five rats.
b) Numbers in parentheses are ratio to the plasma value.
c) Numbers in parentheses are ratio to the tumor value of UFT.
d) Significantly different from the plasma value at $P<0.05$.
e) Significantly different from the plasma value at $P<0.01$.
f) Significantly different from the tumor value with UFT at $P<0.05$.

with tumor cells and the histological appearance of solid tumors in the lung was identical to that of subcutaneously inoculated tumors.

Concentrations of 5-FU in plasma, normal lung tissue and tumor tissue 5-FU levels in the plasma, normal lung tissue and tumor tissue were determined at 2, 6 and 12 h after administration. Six hours after UFT administration the 5-FU level was 90.4±42.4 ng/g in tumor tissue, which was 14 times higher ($P<0.05$) than that in the plasma (6.6±3.9 ng/ml), and it was 63.4±16.7 ng/g in normal lung tissue. 5-FU almost disappeared from the plasma within 12 h. Six hours after BOF-A2 administration, the 5-FU level was 216.8±117.7 ng/g in tumor tissue, 7 times higher ($P<0.05$) than that in the plasma (32.6±19.2 ng/ml). The 5-FU level 12 h after BOF-A2 administration was 129.8±53.0 ng/g in tumor tissue, which was significantly higher ($P<0.01$) than that in the plasma (23.2±7.3 ng/ml). The concentration in tumor tissue was also significantly higher ($P<0.05$) than that in tumors following UFT treatment (129.8±53.0 ng/g and 19.2±11.3 ng/g, respectively) (Table I).

EM-FU and CNDP levels in plasma after oral administration of BOF-A2 As shown in Fig. 2, high EM-FU levels (>1000 ng/ml) and CNDP levels (>100 ng/ml) were maintained for over 12 h.

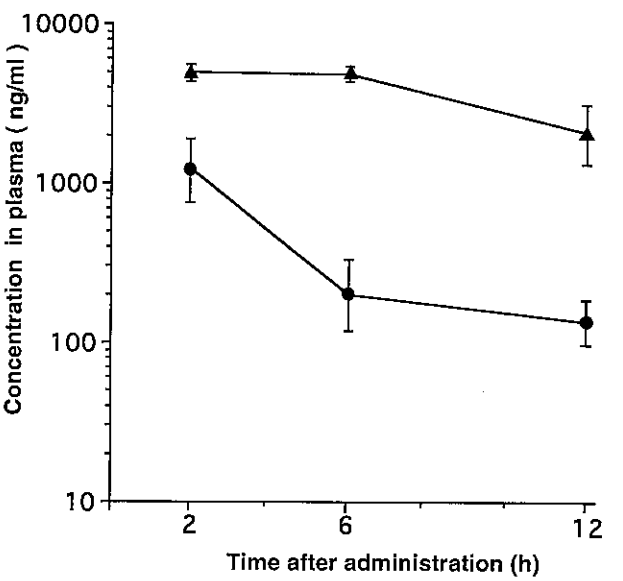

Fig. 2. Concentrations of EM-FU (▲) and CNDP (●) in the plasma after oral BOF-A2 (36 μmol/kg) administration to rats with lung transplanted tumors. Mean±SD (n=5).

DISCUSSION

To compare the levels of 5-FU in lung tumor tissue after administration of BOF-A2 or UFT, we developed a new animal model in which a block of Yoshida sarcoma tissue was transplanted intravenously into the lung of rats. In spite of a fairly low incidence (13/42) of tumor growth, we were able to prepare lung tumors large enough to allow measurement of the drug concentrations. We were also able to confirm that the lung tumors were histologically similar to the original tumor which was transplanted subcutaneously. This lung transplanted tumor model in comparison with the standard subcutaneously transplanted tumor model, is convenient for 5-FU determination in progressed tumor in the lung or for simultaneous evaluation of 5-FU levels in tumor and normal tissue of the same rat.

The dosages of BOF-A2 (36 μmol/kg) and UFT (50 μmol/kg) were chosen on the basis of preliminary studies of 5-FU level in plasma, or tumor growth inhibition dose (ED_{50}). The dosage of BOF-A2 was selected as 36 μmol/kg (ED_{50} for antitumor effect against subcutaneous Yoshida sarcoma). At this dose of BOF-A2, the 5-FU level reached a maximum of 30–50 ng/ml in plasma at 4–6 h. The UFT dose was selected as 50 μmol/kg

since this gave an equivalent 5-FU level in plasma at 1 h. Although this dose of UFT is less than the therapeutic dose of UFT (100–150 μmol/kg), we considered that 5-FU content in lung transplanted tumor could be better compared at equivalent 5-FU levels in plasma. The 5-FU levels 12 h after BOF-A2 administration remained higher in the plasma and especially in the tumor tissue than when UFT was given. The differences in 5-FU levels and in the maintenance of 5-FU levels in tumor tissue after administration of BOF-A2 and UFT probably reflect the inhibitory potency of CNDP or uracil on 5-FU degradation. The inhibitory activity of CNDP on 5-FU degradation is reported to be about 2000 times more potent than that of uracil.[13]

The maintenance of high EM-FU and CNDP levels in the plasma, as well as the potent inhibition of 5-FU degradation, may explain the high 5-FU levels in tumor tissue after BOF-A2 administration. We did not determine BOF-A2 level, because BOF-A2 itself could not be detected in plasma after administration on the basis of preliminary pharmacokinetic data, and the main metabolites have been shown to be 5-FU, EM-FU and CNDP. Levels of both EM-FU and CNDP derived from BOF-A2 were found to remain high for 12 h, suggesting that EM-FU may contribute to the supply of 5-FU in the lung tumor tissue.

We conclude from these results that BOF-A2 administration results in prolonged high levels of 5-FU in lung tumor tissue, and that it may be advantageous as an antitumor agent in humans. However, additional studies are needed, particularly to determine the 5-FU level generated by a therapeutic dose of UFT in lung transplanted tumors and to evaluate 5-FU content or efficacy in subcutaneous Yoshida sarcoma at the doses used in this study.

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