Leukopenia-Inducing Effect of a Combination of a New 5-Fluorouracil (5-FU)-Derived Drug, BOF-A2 (Emitefur), with Other 5-FU-Derived Drugs or BV-araU (Sorivudine) in Rats

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ABSTRACT—BOF-A2 (emitefur: 3-{3-[6-benzoyloxy-3-cyano-2-pyridyloxycarbonyl]benzoyl}-1-ethoxymethyl-5-fluorouracil), a novel 5-FU (5-fluorouracil)-derived drug, was co-administered with other conventional 5-FU-derived drugs or BV-araU [sorivudine: 1- β -D-arabinofuranosyl-(*E*)-5-(2-bromovinyluracil)] for 8 consecutive days to rats. BOF-A2 (6 or 8 mg/kg, p.o.) co-administered with other 5-FU-derived drugs elevated the plasma 5-FU concentration 3- to 23.3-fold and decreased the peripheral white blood cell (WBC). The percentage decreases of WBC by 5-FU (4 mg/kg, i.p.), UFT (16 mg/kg, p.o.), tegafur (FT; 16 mg/kg, p.o.), carmofur (HCFU; 15 mg/kg, p.o.), doxifluridine (5'-DFUR; 16 mg/kg, p.o.) and flucytosine (200 mg/kg, p.o.) were 25.7%, 31.9%, 70.3%, 32.0%, 58.6% and 30.0%, respectively, compared with each drug alone. On the other hand, these phenomena did not occur with BV-araU. These findings can be attributed to the fact that the inhibitory activity of CNDP (3-cyano-2,6-dihydroxypyridine) for 5-FU degradation (IC_{50} : 6.3×10^{-9} M) is potent and 6000 times greater than that of BVU [(*E*)-5-(2-bromovinyl) uracil], another inhibitor of 5-FU degradation.

Keywords: Emitefur (BOF-A2), 5-Fluorouracil-derived drug, Sorivudine (BV-araU), Leukopenia

Uracil and 5-substituted uracil derivatives accelerate the antitumor activity of 5-fluorouracil (5-FU)-derived drugs by inhibiting 5-FU degradation (1–4). As shown in Fig. 1, CNDP (3-cyano-2,6-dihydroxypyridine) is a more potent inhibitor of 5-FU degradation than uracil (5). BOF-A2 (3-{3-[6-benzoyloxy-3-cyano-2-pyridyloxycarbonyl]benzoyl}-1-ethoxymethyl-5-fluorouracil), a novel 5-FU-derived antineoplastic drug, was developed by conjugating CNDP and EM-FU (1-ethoxymethyl-5-fluorouracil), a 5-FU precursor that steadily releases 5-FU via liver microsomes (6).

Consequently, due to the persistence of 5-FU in the plasma or tumor tissue compared with other 5-FU-derived drugs, BOF-A2 has potent antitumor activity against experimental tumors and human cancer xenografts (7, 8). Thus, BOF-A2 is used more than other 5-FU-derived drugs in lung cancer patients (9).

Cancer patients are rarely treated with one drug alone, and most anticancer drugs are co-administered with other agents such as antiviral drugs, hormones and/or steroids. A combination of the antiviral drug BV-araU [1- β -D-

arabinofuranosyl-(*E*)-5-(2-bromovinyluracil)] with 5-FU-derived anticancer drugs causes severe leukopenia (10). This condition is also induced by a high 5-FU dosage or a high plasma 5-FU concentration (11–13). The BVU [(*E*)-5-(2-bromovinyl) uracil] metabolite of BV-araU elevates the 5-FU concentration in the blood by inhibiting dihydropyrimidine dehydrogenase (DPDase) (14). This acute elevation in the 5-FU concentration suggests that catabolism inhibition by drug-interactions may increase adverse side effects in humans. Though it is unlikely that BOF-A2 will be combined with other 5-FU-derived anticancer drugs, this danger cannot be ignored.

We studied the effect of BOF-A2 in combination with other 5-FU-derived drugs and BV-araU on the 5-FU concentration in the blood and upon leukopenia in rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley strain rats at the age of 6–8 weeks were purchased from Clea Japan, Inc. (Tokyo) for

Fig. 1. Proposed metabolic pathway of BOF-A2.

repeated administration and from Charles River Japan, Inc. (Kanagawa) for single administration. The rats were housed in a temperature ($24 \pm 2^\circ\text{C}$)- and humidity ($60 \pm 10\%$)-controlled room. The experiments were begun after a preliminary housing period of one week.

Drugs

BOF-A2 was synthesized at the Second Factory of Otsuka Pharmaceutical Co., Ltd. (Tokushima). The following were obtained from commercial sources: FT and UFT (Taiho Pharmaceutical Co., Ltd., Tokyo), 5-FU (Daikin Kogyo Co., Ltd., Tokyo), 5'-DFUR and flucytosine (Nippon Roche, Tokyo) and HCFU (Yamanouchi Pharmaceutical Co., Ltd., Tokyo). BV-araU was provided by Yamasa Shoyu Co., Ltd., Chiba. The method of BOF-A2 synthesis is described elsewhere (6), and the structures of all drugs applied are shown in Fig. 2. All other chemicals used were of analytical grade. All drugs except for 5-FU were suspended in 1% HPMC (hydroxypropyl methyl cellulose) by stirring for 10 to 30 min. 5-FU was dissolved in saline for repeated administration or in 1% HPMC for single administration.

Effect of repeated co-administration of BOF-A2 with other 5-FU-derived drugs (5-FU, UFT, FT, 5'-DFUR, HCFU and flucytosine) on white blood cell (WBC) and plasma 5-FU concentration

The doses of the drugs given in combination were set at the respective minimum doses of 4 mg/kg, i.p. for 5-FU; 6 mg/kg, p.o. for BOF-A2 and UFT; 15 mg/kg, p.o. for HCFU; and 16 mg/kg, p.o. for FT and 5'-DFUR. Each 5-FU-derived drug was administered once daily for 8 days alone or in combination with BOF-A2 to 7 normal rats per group. The rats were weighed before each administration. Five milliliters of heparinized blood per rat was collected 1 hr after the final administration, and peripheral WBC and plasma 5-FU concentrations were measured. For the 8-day consecutive combination treatment of BOF-A2 (8 mg/kg, p.o.) and flucytosine (200 mg/kg, p.o.), 5 ml of blood per rat was collected 2 hr after the final administration.

Effect of single co-administration of BOF-A2 with 5-FU or UFT

The doses were 20 mg/kg, p.o. for BOF-A2 and UFT and 4.7 mg/kg, p.o. for 5-FU (equimolar to 20 mg/kg of BOF-A2). The drugs alone or in combination with BOF-A2 were orally administered once to 3 rats at each point described below. Five milliliters of heparinized blood per rat was collected at 0.17, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 12 and 24 hr after the combination of BOF-A2 and 5-FU, and at 0.5, 1, 2, 4, 6, 8 and 12 hr after the combination of BOF-A2 and UFT. The AUC (area under the curve, ng·hr/ml) for the plasma 5-FU concentration was deter-

Fig. 2. Structural formula of test compounds.

mined by means of the trapezoidal rule.

Effect of repeated co-administration of BV-araU with BOF-A2 or UFT on WBC and plasma 5-FU concentration

The doses of oral BV-araU used in combination were 20 and 100 mg/kg, and those of oral BOF-A2 and UFT were 10, 20 and 40 mg/kg. BV-araU was given once daily for 8 days alone or in combination with BOF-A2 or UFT to 7 rats. The rats were weighed before each administration. Five milliliters of heparinized blood per rat was collected 1 hr after the final administration, and peripheral WBC and plasma 5-FU concentration were measured.

Effect of single co-administration of BV-araU with BOF-A2 or 5-FU

The dose of oral BV-araU was set at 20 mg/kg and that of oral BOF-A2 and 5-FU combined with BV-araU at 20 mg/kg and 4.7 mg/kg (equimolar to 20 mg/kg of BOF-A2), respectively. The drugs were orally administered once to 3 rats at each point described below. Five milliliters of heparinized blood per rat was collected at 0.17, 0.33, 0.5, 0.75, 1, 2, 4, 6 and 8 hr after the co-administration of BV-araU and 5-FU, and at 0.5, 1, 2, 4, 6, 8, 12 and 24 hr after the co-administration of BV-araU and BOF-A2. Plasma 5-FU, EM-FU and CNDP concentrations were measured by HPLC (high performance liquid

chromatography), and the AUC for each metabolite was determined by means of the trapezoidal rule.

Determination of plasma 5-FU concentration by GC-MS

The plasma 5-FU concentration was measured 1 or 2 hr after the final administration of BOF-A2 and the other 5-FU-derived drugs by a partially modified version of the GC-MS (gas chromatography-mass spectrometer) procedure reported by Kubo et al. (15). Five milliliters of heparinized blood collected through the inferior vena cava was centrifuged at 3,000 r.p.m. for 10 min. The supernatant was kept at -20°C until the plasma 5-FU concentration was determined. Distilled water (2 ml) containing 0.1 μg of internal standard (1,3-bis- ^{15}N -5-FU), 50 μl of 5 N hydrochloric acid and 40 ml of chloroform-methanol (50:1) was added to 250 μl of plasma and shaken. To the aqueous phase, 40 ml of ethyl acetate was added to extract 5-FU. The organic phase was evaporated to dryness under a stream of N_2 gas. Silylation of the 5-FU was performed as follows: A mixture of 1 ml of *N,O*-bis-(trimethylsilyl) trifluoroacetamide (BSTFA), 1 ml of pyridine and 2 ml of toluene was added to the test tube and heated for 20 min at 80°C . The ion peak ($M/Z=274.4$, 276.4) corresponding to the molecular ion peak of silylated 5-FU or silylated internal standard was monitored by GC-MS.

Determination of plasma 5-FU EM-FU and CNDP by HPLC

The plasma 5-FU concentrations after consecutive co-administration of BOF-A2 with BV-araU and after a single administration of BOF-A2 with 5-FU or UFT were determined by HPLC. Five milliliters of heparinized blood collected through the inferior vena cava of each rat was quickly centrifuged for plasma preparation.

We extracted 5-FU as follows: An internal standard comprised of 5-bromouracil (0.01 ml), 1 ml of saturated ammonium sulfate and 5 ml of CHCl_3 was added to 1 ml of plasma sample and shaken. To extract the 5-FU, ethyl acetate (3 ml) was added to the aqueous phase; this extraction by ethyl acetate was repeated once more. The

organic phase was evaporated to dryness under a stream of air and then dissolved in 100 μl of water for HPLC. The conditions for HPLC were as follows: mobile phase of 0.1 N acetic acid, 280-nm UV detection wave, YMC A-303 reverse-phase column (4.6×150 mm) (YMC Co., Ltd., Kyoto).

CNDP was extracted as follows: aliquots of an internal standard comprised of 5-carbamoyl-3-fluoro-2,6-dihydroxypyridine (synthesized by the Fujii Memorial Research Institute, Otsuka Pharmaceutical Co., Ltd., Ohtsu) and 400 μl of CH_3CN were added to 100 μl of the plasma sample, and then the mixture was separated by centrifugation. The supernatant was evaporated to dryness under a stream of air and then dissolved in 100 μl of

Fig. 3. Effect of combining 5-FU-derived drugs with BOF-A2 on the white blood cell (WBC). BOF-A2 (6 mg/kg, p.o.) combined with other 5-FU-derived drugs (5-FU, 4 mg/kg, i.p.; UFT, 6 mg/kg, p.o.; FT, 16 mg/kg, p.o.; HCFU, 15 mg/kg, p.o.; 5'-DFUR, 16 mg/kg, p.o.) was administered to SD strain rats once daily for 8 consecutive days, and peripheral WBC was counted 1 hr after the final dose. Values are expressed as means $\pm$ S.D. of 7 rats in each group. NS: Not significant. * $P < 0.05$, ** $P < 0.01$ (significant difference between combination and non-combination groups by Dunnett's test).

the HPLC mobile phase. The conditions for HPLC were as follows: mobile phase of CH_3CN –0.1 M acetate buffer (1:99) (pH 5.0), 330-nm (Ex) and 380-nm (Em) detection wave, YMC A-302 reverse-phase column (4.6×150 mm) (YMC Co., Ltd.).

EM-FU was extracted as follows: aliquots of an internal standard comprised of 1-ethoxymethylthymine (synthesized by the Fujii Memorial Research Institute, Otsuka Pharmaceutical Co., Ltd.) and 1 ml of saturated ammonium sulfate were added to 1 ml of the plasma sample; and this mixture was shaken with 5 ml of CHCl_3 . The organic phase was evaporated to dryness under a stream of air and then dissolved in 100 μl of 30% methanol. The conditions for HPLC were as follows: mobile phase of

CH_3CN – acetic acid – H_2O (10:1:89), 280-nm UV detection wave, YMC A-302 reverse-phase column (4.6×150 mm) (YMC Co., Ltd.). The extraction of BVU, which is a metabolite of BV-araU, and the HPLC analysis were performed under the conditions used for EM-FU.

Determination of WBC

The peripheral WBC in blood collected through the inferior vena cava using a heparinized syringe was counted in an automatic microcell counter equipped with electrical resistor (Sysmex F-800; Toa Medical Electronic Co., Ltd., Tokyo).

Fig. 4. Effect of combining 5-FU-derived drugs with BOF-A2 on the plasma 5-FU concentration. BOF-A2 (6 mg/kg, p.o.) combined with other 5-FU-derived drugs (5-FU, 4 mg/kg, i.p.; UFT, 6 mg/kg, p.o.; FT, 16 mg/kg, p.o.; HCFU, 15 mg/kg p.o., 5'-DFUR, 16 mg/kg, p.o.) was administered to SD strain rats once daily for 8 consecutive days, and plasma was collected 1 hr after the final dose to determine the plasma 5-FU concentration. Values are expressed as means $\pm$ S.D. of 7 rats in each group. ** $P < 0.01$ (significant difference between combination and non-combination groups by Dunnnett's test).

Fig. 5. Effect of combining flucytosine with BOF-A2 on the white blood cell (WBC) and the plasma 5-FU concentration. BOF-A2 (8 mg/kg, p.o.) combined with flucytosine (200 mg/kg, p.o.) was administered to SD strain rats once daily for 8 consecutive days, and blood and plasma were collected 2 hr after the final dose to determine the peripheral WBC count and plasma 5-FU concentration. Values are expressed as means $\pm$ S.D. of 7 rats in each group. ** $P < 0.01$ (significant difference between combination and non-combination groups by Dunnett's test).

Inhibitory activity of each inhibitor on 5-FU degradation

The inhibitory activity of each inhibitor (CNDP, uracil, BVU and BV-araU) on 5-FU degradation was determined in normal rat liver tissue homogenates. The liver of SD-rats (6-weeks-old) was excised under anesthesia and immediately homogenized (20% W/V) in 0.25 M sucrose containing 0.5 mM EDTA and 5 mM β -mercaptoethanol. The homogenate was centrifuged for 60 min at $10,500 \times g$, and the supernatant was dialyzed overnight against 10 mM potassium phosphate buffer (pH 7.5) for use as the crude enzyme. The reaction mixture containing the crude enzyme (0.05% as liver weight), 45 mM potassium phosphate buffer (pH 7.5), 50 mM nicotinamide, 5

mM ATP, 25 mM NaF, 1 mM NADPH, 10 μ M [3 H]5-FU (1.02 μ Ci/ml) and serial concentrations of each inhibitor was incubated for 30 min at 37°C. The radioactivity was measured as described before (16).

Statistical analyses

The peripheral WBC count and plasma 5-FU concentration after consecutive administrations are expressed as means $\pm$ S.D. Statistical analysis between combination groups was performed by dispersed analysis followed by Dunnett's test or Student's *t*-test. The *P* value of statistically significant differences was set at $P < 0.05$.

Table 1. Pharmacokinetic parameters of 5-FU in plasma after a single oral co-administration of BOF-A2 with other 5-FU-derived drugs

Drug	Dose (mg/kg)	Admin. route	5-FU		
			T_{max} (hr)	C_{max} (ng/ml)	$AUC_{0-12 \text{ hr}}$ (ng·hr/ml)
BOF-A2	20	p.o.	8	335	2712
5-FU	4.7	p.o.	0.17	595	114
UFT	20	p.o.	1	849	1747
BOF-A2 + 5-FU	20 + 4.7	p.o. + p.o.	0.33	3463	6403
BOF-A2 + UFT	20 + 20	p.o. + p.o.	4	2143	14836
BOF-A2 + BV-araU	20 + 20	p.o. + p.o.	6	377	2147
5-FU + BV-araU	4.7 + 20	p.o. + p.o.	0.17	923	197

BOF-A2 (20 mg/kg), UFT (20 mg/kg), 5-FU (4.7 mg/kg) and BV-araU (20 mg/kg) were orally co-administered once to SD-strain rats, and the plasma 5-FU concentrations were measured by HPLC as described in Materials and Methods. Values are expressed as means of 3 rats in each group.

RESULTS

Effect of repeated co-administration of BOF-A2 with 5-FU-derived drugs on WBC and the plasma 5-FU concentration

As shown in Fig. 3, the peripheral WBC was significantly decreased by BOF-A2 combined with 5-FU, UFT, FT, HCFU and 5'-DFUR to levels of 25.7% ($P < 0.01$), 31.9%, 70.3% ($P < 0.01$), 32.0% ($P < 0.01$) and 56.8% ($P < 0.01$), respectively, compared with each drug alone. As shown in Fig. 4, the plasma 5-FU concen-

tration after combination of BOF-A2 with the other 5-FU-derived drugs was remarkably elevated to 3–23.3 times that with each 5-FU-derived drug alone.

Effect of repeated co-administration of BOF-A2 with flucytosine on WBC and the plasma 5-FU concentration

As shown in Fig. 5, the combination of BOF-A2 and flucytosine significantly decreased the peripheral WBC by 31.0% ($P < 0.01$) and 30.0% ($P < 0.01$), respectively, compared with BOF-A2 and flucytosine alone. The combination of these drugs markedly increased the plasma 5-

Fig. 6. Effect of BV-araU with BOF-A2 on the white blood cell (WBC) and the plasma 5-FU concentration. BOF-A2 (10, 20, 40 mg/kg, p.o.) combined with BV-araU (20, 100 mg/kg, p.o.) was administered to SD strain rats once daily for 8 consecutive days, and peripheral WBC and plasma 5-FU were measured after the final dose. Values are expressed as means $\pm$ S.D. of 7 rats in each group. NS: not significant. * $P < 0.05$, ** $P < 0.01$ (significant difference between combination and non-combination groups by Dunnett's test or the *t*-test).

FU concentration 7.3 times compared with flucytosine alone.

Effect of single co-administration of BOF-A2 with 5-FU or UFT

As shown in Table 1, at 3463 ng/ml, the C_{max} after the combined administration of BOF-A2 (20 mg/kg) with 5-FU (4.7 mg/kg) was 5.8 times that after 5-FU alone (595 ng/ml). The $AUC_{0-12\text{ hr}}$ of 6403 ng·hr/ml after combined administration was 2.3 times the sum (2826 ng·hr/ml) of the $AUC_{0-12\text{ hr}}$ of each drug alone.

On the other hand, at 2143 ng/ml, the C_{max} after combination of BOF-A2 (20 mg/kg) with UFT (20 mg/kg)

was 2.5-fold higher than that after UFT alone (849 ng/ml). The $AUC_{0-12\text{ hr}}$ of 14836 ng·hr/ml after combined administration was 3.3 times the sum (4459 ng·hr/ml) of the $AUC_{0-12\text{ hr}}$ of each drug alone.

Effect of repeated co-administration of BV-araU with BOF-A2 or UFT on WBC and plasma 5-FU concentration

BV-araU at doses of 20 or 100 mg/kg was co-administered once daily for 8 days with BOF-A2 or UFT at doses of 10, 20 or 40 mg/kg. As shown in Figs. 6 and 7, the peripheral WBC and plasma 5-FU concentration were measured 1 hr after the final co-administration.

Fig. 7. Effect of BV-araU with UFT on the white blood cell (WBC) and the plasma 5-FU concentration. UFT (10, 20, 40 mg/kg, p.o.) combined with BV-araU (20 mg/kg, p.o.) was administered to SD strain rats once daily for 8 consecutive days, and peripheral WBC and plasma 5-FU were measured 1 hr after the final dosing. Values are expressed as means $\pm$ S.D. of 7 rats in each group. ** $P < 0.01$, *** $P < 0.001$ (significant difference between combination and non-combination groups by Dunnett's test or the t -test).

Fig. 8. Inhibitory effect of CNDP (●), uracil (○), BVU (△) and BV-araU (▲) on 5-FU degradation in rat liver. The metabolites (F-β-Ala and FUPA) of 5-FU were measured by thin-layer chromatography after incubation for 30 min at 37°C with each inhibitor using 0.5% rat liver homogenate.

Diarrhea occurred on day 4 and two rats died on the final day of the combined administration of UFT (40 mg/kg) and BV-araU (20 mg/kg), but not after the combined BOF-A2 with BV-araU.

The co-administration of BOF-A2 (10, 20 or 40 mg/kg) with BV-araU (20 or 100 mg/kg) did not enhance the decrease in peripheral WBC at any dose tested. However, the peripheral WBC after co-administration of UFT at 10, 20 or 40 mg/kg with BV-araU (20 mg/kg) decreased by 52.3% ($P < 0.01$), 81.8% ($P < 0.01$) and 80.8% ($P < 0.01$) respectively, compared with that after UFT alone.

The plasma 5-FU concentration was not elevated after the co-administration of BOF-A2 (20 mg/kg) with BV-araU at 100 mg/kg and conversely declined after the co-administration of BOF-A2 (20 mg/kg) with BV-araU at 20 mg/kg. However, co-administration of UFT with BV-araU (20 mg/kg) significantly elevated the plasma 5-FU concentration to 226 ng/ml ($P < 0.01$) for UFT at 10 mg/kg and to 434 ng/ml ($P < 0.01$) for UFT at 20 mg/kg compared with UFT alone (15 ng/ml at 10 mg/kg and 241 ng/ml at 20 mg/kg). Co-administration of a high dose (40 mg/kg) of UFT with BV-araU (20 mg/kg) did not elevate the plasma 5-FU concentration compared with UFT alone.

Effect of single co-administration of BV-araU with BOF-A2 or 5-FU

Table 1 shows the pharmacokinetic parameters when BV-araU (20 mg/kg) was administered 1 hr before BOF-

A2 (20 mg/kg) or 5-FU (4.7 mg/kg). The $C_{\max}$ and $AUC_{0-12 \text{ hr}}$ of 5-FU after co-administration of BOF-A2 with BV-araU were 377 ng/ml and 2147 ng·hr/ml, respectively. These were 1.1 and 0.8 times the respective values after BOF-A2 alone, indicating that the plasma 5-FU concentration was not elevated by co-administering BV-araU with BOF-A2. The $C_{\max}$ and $AUC_{0-24 \text{ hr}}$ of EM-FU were unchanged. The $C_{\max}$ of CNDP was 1198 ng/ml at 4 hr. The $C_{\max}$ of BVU, a metabolite of BV-araU, was 248 ng/ml at 8 hr, and BVU persisted for as long as CNDP, although at a lower concentration. However, the $C_{\max}$ and $AUC_{0-12 \text{ hr}}$ of the plasma 5-FU after co-administration of 5-FU with BV-araU were 923 ng/ml and 197 ng·hr/ml, respectively, which were 1.6 and 1.7 times the respective values obtained after the administration of 5-FU alone.

Inhibitory activity of CNDP, uracil, BVU and BV-araU on 5-FU degradation

As shown in Fig. 8, The IC_{50} values of CNDP, uracil, BVU and BV-araU on 5-FU degradation were 6.3×10^{-9} , 6.4×10^{-6} , 3.7×10^{-5} M and over 10^{-4} M, respectively. CNDP was potently inhibitory, whereas the inhibitory activity of BVU was one-sixth that of uracil, and BV-araU itself was not inhibitory even at 10^{-4} M.

DISCUSSION

BOF-A2, which is a new 5-FU-derived antineoplastic drug, rapidly releases EM-FU, a 5-FU precursor, and CNDP, a potent inhibitor of 5-FU degradation, after oral administration. EM-FU is further metabolized to 5-FU via liver microsomes. CNDP is simultaneously distributed in the body and potently inhibits DPDase, an enzyme found in normal liver and human lung cancer tissues that catalyze 5-FU degradation. As a result, BOF-A2 shows a potent antitumor activity (5, 9, 16, 17).

Because BOF-A2 contains CNDP, a potent inhibitor of the catabolic degradation of 5-FU, we considered that its co-administration with other 5-FU-derived antineoplastic drugs could invoke severe side effects.

We studied the effects of the co-administration of BOF-A2 with the other 5-FU-derived antineoplastic drugs, 5-FU, UFT, FT, HCFU and 5'-DFUR, and the 5-FU-derived antifungal drug flucytosine on the plasma 5-FU concentration and number of peripheral WBC in rats.

When BOF-A2 and other 5-FU-derived drugs were consecutively co-administered at the standard clinical doses, WBC was markedly decreased with significant differences compared with each drug alone. One reason for these effects is likely to be the significant elevation of the 5-FU blood concentration compared with each drug alone. This hypothesis was also supported by the increase

in the AUC after a single co-administration of BOF-A2 with 5-FU or UFT.

Furthermore, BV-araU has been developed as an antiviral drug (18), and severe leukopenia has been induced by its co-administration with 5-FU-derived anticancer drugs. It was thought that the mechanism behind this phenomenon was the inhibitory activity of BVU, which is a metabolite of BV-araU (18, 19), upon 5-FU degradation. After co-administration of UFT (10 or 20 mg/kg) and BV-araU (20 mg/kg), there was a marked decrease in peripheral WBC, accompanied by an elevated plasma 5-FU concentration. This phenomenon is considered to be based on two mechanisms: the persistence of BVU in the blood and the irreversible inhibition of 5-FU catabolism by BVU, even though its inhibitory activity is only one-sixth that of uracil (14, 19, 20). However, co-administration of BOF-A2 (10, 20 or 40 mg/kg) with BV-araU (20 mg/kg) did not decrease peripheral WBC. This finding was attributable to the fact that the inhibitory potency of CNDP for the degradation of 5-FU is 6000 times greater than that of BVU. As a result, the co-administration of BV-araU did not additionally inhibit 5-FU degradation.

Thus, although leukopenia was increased and the blood 5-FU concentration was elevated after co-administration of BOF-A2 with other 5-FU-derived drugs, none of the marked changes caused by the co-administration of UFT with BV-araU were seen after the co-administration of BOF-A2 with BV-araU.

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