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ENHANCING EFFECT OF BROMOVINYLDEOXYURIDINE ON ANTITUMOR ACTIVITY OF 5′-DEOXY-5-FLUOROURIDINE AGAINST ADENOCARCINOMA 755 IN MICE

CORRELATION WITH PHARMACOKINETICS OF PLASMA 5-FLUOROURACIL LEVELS

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Abstract—5′-Deoxy-5-fluorouridine (DFUR), whether or not combined with (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) was pursued in BDF_1 mice from both a pharmacokinetic viewpoint, following a single oral dose administration, and an anticancer viewpoint, following 5 daily oral doses in mice inoculated subcutaneously with adenocarcinoma 755 tumor cells. Half-life ($t_{1/2}$) values for the elimination of DFUR and 5-fluorouracil (5-FU) from plasma following DFUR (100 mg/kg) administration were about 0.80 and 0.39 hr, respectively. Plasma 5-FU AUC (area under the curve) values following oral DFUR (100 mg/kg) was 0.224 μg·hr/ml. If DFUR (100 mg/kg) was combined with BVDU (10 mg/kg) the $t_{1/2}$ and AUC values for 5-FU increased from 0.39 to 1.24 hr, and from 0.224 to 1.699 μg·hr/ml, respectively. Thus, BVDU significantly increased the plasma levels of 5-FU. It had no effect on the plasma levels of DFUR. At 100 mg/kg, DFUR did not show a significant antitumor activity. At 500 mg/kg it effected a 90% inhibition in tumor growth. When combined with BVDU (10 mg/kg), DFUR at 100, 200 and 300 mg/kg reduced tumor growth by 96, 100 and 100%, respectively. The antitumor activity achieved by DFUR, in the presence or absence of BVDU, correlated highly significantly with the AUC values for plasma 5-FU.

5′-Deoxy-5-fluorouridine (DFUR) is a prodrug of 5-fluorouracil (5-FU) and currently used in the clinic (in Japan) as an oral anticancer drug. As compared to 5-FU, DFUR has a similar activity spectrum but a higher therapeutic index against murine tumors [1–4]. The antitumor activity of DFUR depends on its intracellular activation to 5-FU by pyrimidine nucleoside phosphorylases [3–5]. These enzymes are present in some tumors, liver and small intestine [1, 3–5]. 5-FU generated in these tissues is released and distributed via the bloodstream to other tissues, i.e. tumors which lack pyrimidine nucleoside phosphorylase activity and therefore cannot activate DFUR.

5-FU, however, is rapidly degraded in the liver [6] through a reductive pathway involving dihydrothymine (dihydrouracil) dehydrogenase [7]. A single phase elimination of 5-FU from plasma occurs with $t_{1/2}$ values of 8–20 min following intravenous (i.v.) bolus injection [8]. When 5-FU is combined with (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU), the anti-tumor activity of 5-FU is potentiated, its plasma clearance slowed down and its half-life prolonged, especially following oral administration [9, 10]. The reason for the enhancing effect of BVDU on the antitumor activity of 5-FU is that, following degradation of BVDU by pyrimidine nucleoside phosphorylase(s) to BVU [(*E*)-5-(2-bromovinyl)uracil], the latter prevents the degradation of 5-FU at the dihydrothymine dehydrogenase level [11]. The present studies were undertaken to determine whether BVDU also potentiates the antitumor activity of oral DFUR and, if so, to provide a pharmacokinetic explanation for the enhancing effect of BVDU on the antitumor activity of DFUR.

MATERIALS AND METHODS

Drugs. DFUR was provided by Nippon Roche, Kamakura, Japan. BVDU was synthesized essentially as described by Jones *et al.* [12].

Animals. Groups of six (for determining antitumor effect) or three (for assaying plasma drug levels) male BDF_1 mice with body weight of 21–23 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were housed in plastic cages with woodchip bedding, and received a CA-1 pellet diet (CLEA Japan, Inc., Tokyo, Japan) and water *ad libitum*. All experiments were performed in the animal laboratory at a controlled temperature of 25°.

Plasma drug concentration determinations. Groups of three mice were given DFUR and BVDU at the indicated doses in 0.1 ml physiological saline by the oral route (perorally, p.o.). Blood samples (0.6–1.0 ml) were collected in heparinized tubes at 0.25, 0.5, 1, 2, 3, 4, 5 and 6 hr following treatment, and centrifuged immediately after collection. The plasma supernatants were harvested and frozen at −20° until assayed. Plasma samples were adjusted with distilled water to a total volume of 1 ml, and 0.2 ml of 0.5 M

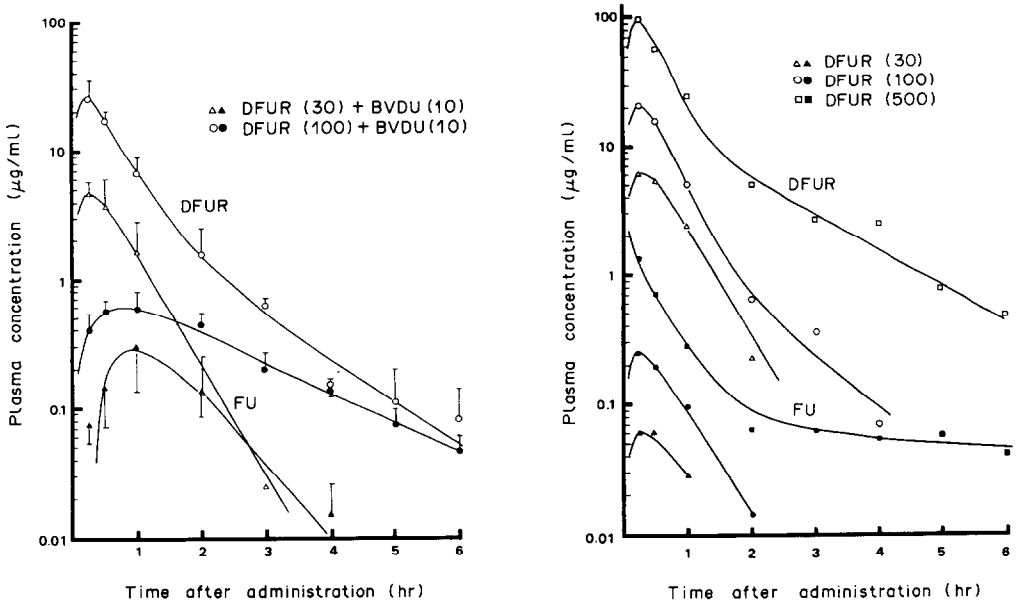

Fig. 1. Plasma DFUR and 5-FU concentration profiles following oral administration of DFUR without or with BVDU to mice. Open symbols refer to DFUR concentrations and closed symbols refer to 5-FU concentrations, the latter being derived from DFUR.

Table 1. Pharmacokinetic parameters of DFUR in mice after oral administration of DFUR without or with BVDU

Dose mg/kg		AUC	Vc/F*	ka‡	k_{12}‡	k_{21}‡	k_{el}‡	$t_{1/2}$§
DFUR	BVDU	μg·hr/ml	ml/kg	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr
30	—	5.64	2659.5†	5.370	—	—	1.999	0.35
100	—	15.84	2076.5	4.651	0.352	1.101	3.041	0.80
300	—	43.07	3164.3	5.087	0.438	0.629	2.201	1.39
500	—	75.00	2236.9	4.882	1.069	0.901	2.980	1.12
1000	—	215.20	2061.8	3.483	0.287	0.253	2.254	3.13
30	10	3.95	3802.6†	6.261	—	—	1.997	0.35
100	10	20.65	2559.7	9.904	0.267	0.932	1.892	0.92

*F: bioavailability. Vc: volume of central compartment.
† Apparent volume of distribution.
‡ Absorption, intercompartmental transfer and elimination rate constants.
§ Terminal half-life.

NaH_2PO_4 buffer and 8 ml of ethyl acetate were added. After extraction and centrifugation, the organic layer was transferred to other tubes and evaporated *in vacuo* at 35°. The residue was dissolved in distilled water, in the same volume as originally present as plasma, and 25–100 μl samples were injected into an HPLC (Tosoh, Tokyo, Japan) equipped with a Hibar prepacked column, LiChrosorb RP-18 (5 μm; Cica-Merck, Tokyo, Japan) and Tosoh Model UV-8000 UV–VIS absorbance detector with a 260-nm filter.

Resolution of DFUR and 5-FU. DFUR and 5-FU were separated by using 2% methanol for 10 min, a linear gradient from 2% methanol to 15% methanol–sodium acetate buffer (10 mM, pH 4.0) for 5 min, and a 15% methanol–sodium acetate buffer for 15 min. The flow rate was set at 0.7 ml/min. The retention times of DFUR and 5-FU were 24.9 and 7.6 min, respectively.

Antitumor activity. Mice were inoculated subcutaneously (s.c.) on day 0 with adenocarcinoma 755 tumor cells (20 mg/mouse). The tumors had been maintained by s.c. transfer every 12 days into syngeneic C57BL/6 mice kept in our laboratory at the National Cancer Center Research Institute, Tokyo, Japan. Beginning 24 hr after tumor cell inoculation, the compounds were administered p.o. daily for five consecutive days. Tumor weight was determined on day 11.

Antitumor activity was evaluated by calculating the ratio of the average tumor weight in the treated groups to that in the control group [T/C (%)].

Pharmacokinetic analysis of DFUR. Plasma DFUR concentrations following oral administration of DFUR at a dose of 30 mg/kg with and without BVDU (10 mg/kg) were fitted to a 1-compartment model with first order absorption with the iterative weighed nonlinear least-square regression program MULTI [13].

Table 2. Pharmacokinetic parameters of 5-FU in mice after oral administration of DFUR without or with BVDU

Dose mg/kg		$t_{1/2}$	AUC*	AUC($Cp < 0.5$)†	AUC ($Cp < 0.4$)†	AUC($Cp < 0.3$)†	AUC($Cp < 0.2$)†
DFUR	BVDU	hr	μg·hr/ml	μg·hr/ml	μg·hr/ml	μg·hr/ml	μg·hr/ml
30	—	0.45	0.0631	0.0631	0.0631	0.0631	0.0631
100	—	0.39	0.2238	0.2238	0.2238	0.2238	0.2175
300	—	3.82	0.5766	0.5766	0.5756	0.5548	0.4975
500	—	7.41	1.5202	1.2888	1.2166	1.1308	1.0199
1000	—	3.40	3.2831	1.7583	1.5901	1.4120	1.2238
30	10	0.43	0.4531	0.4531	0.4531	0.4531	0.4531
100	10	1.24	1.6990	1.6379	1.4824	1.2678	1.0039

* AUC from time to the infinite time.
† AUC under the concentration x.

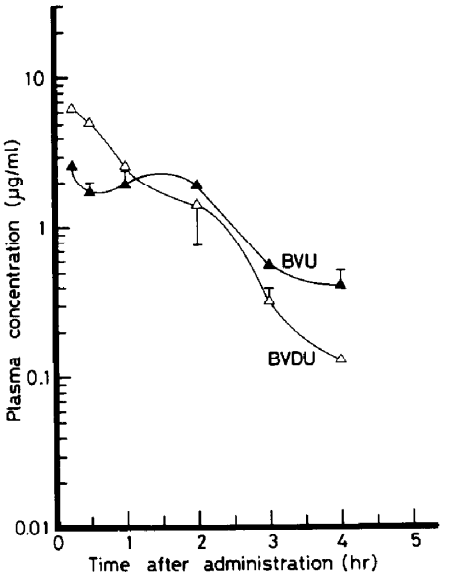

Fig. 2. Plasma BVDU and BVU concentration profiles following oral administration of BVDU (10 mg/kg).

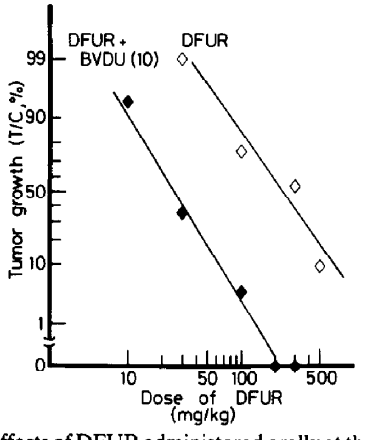

Fig. 3. Effects of DFUR administered orally at the indicated doses, combined with or without oral BVDU (10 mg/kg) on the growth of adenocarcinoma 755 in BDF_1 mice. Treatment was started one day after tumor cell inoculation and continued for five consecutive days. Tumor weight was determined on day 11. There were six mice per group.

Other concentration–time data were analyzed by a 2-compartment model using MULTI with reciprocal concentration weighing. Since bioavailability (F) of DFUR was not determined in this study, the volume of distribution divided by F was calculated.

Elimination half-life ($t_{1/2}$) was calculated by the following equation:

$$t_{1/2} = \ln 2/\lambda$$

where λ is an elimination rate constant. AUC was calculated by integration of the fitted line to the infinite time.

Pharmacokinetic analysis of 5-*FU.* $T_{1/2}$ of 5-FU following oral administration of DFUR was determined by linear regression of the terminal log-linear portion of 5-FU concentration versus time. AUC from time

Table 3. Intercepts, slopes and correlation coefficients of regression lines for inhibition of tumor growth versus AUC

	A*	B*	r†	P‡
AUC	18.60	48.93	0.9103	0.0117
AUC($Cp < 0.5$)	17.22	54.24	0.9137	0.0108
AUC($Cp < 0.4$)	14.93	60.76	0.9256	0.0081
AUC($Cp < 0.3$)	11.49	71.63	0.9422	0.0049
AUC($Cp < 0.2$)	7.12	89.33	0.9593	0.0024

* Statistical fit is to the equation:
Inhibition of tumor growth (%) = $A + B \times$ AUC.
† Correlation coefficient.
‡ Level of significance.

(Fig. 1) or pharmacokinetic parameters (Table 1) of DFUR.

Pharmacokinetics of plasma 5-FU following oral administration of DFUR without or with BVDU

When DFUR was administered to mice at a dose of 30 or 100 mg/kg, no 5-FU could be detected in the plasma 3 hr later. If, however, DFUR at 30 or 100 mg/kg was combined with BVDU (10 mg/kg), a marked prolongation in the plasma 5-FU levels was observed (Fig. 1). AUC and $t_{1/2}$ values of 5-FU are presented in Table 2. The plasma 5-FU half-life following combined DFUR (100 mg/kg) and BVDU (10 mg/kg) administration was 1.24 hr, whereas that after DFUR (100 mg/kg) administration alone was 0.39 hr. Thus, disappearance of 5-FU from plasma after oral administration of DFUR was significantly decreased following co-administration of BVDU. The AUCs of 5-FU following administration of DFUR at 100 and 500 mg/kg were 0.224 and 1.52 μg·hr/ml, respectively. The AUC of 5-FU following combination of DFUR (100 mg/kg) with BVDU was 1.70 μg·hr/ml, thus similar to the AUC value obtained with DFUR if used alone at 500 mg/kg (maximum tolerated dose) (Table 2). The plasma concentration generated following the oral administration of BVDU (10 mg/kg) remained above 0.3 μg/ml during the whole observation period (Fig. 2).

Table 4. Therapeutic index of DFUR combined or not with BVDU

	ED_{50}* (mg/kg/day)	LD_{50}* (mg/kg/day)	Therapeutic index†
DFUR	235	750	3.2
DFUR + BVDU (10 mg/kg/day)	26	360	13.8

* ED_{50} (50% effective dose) corresponded to the dose required to achieve a T/C value of 50%. LD_{50} corresponded to the 50% lethal dose. The compounds were administered p.o. daily for 5 consecutive days.
† Therapeutic index: LD_{50}/ED_{50}.

zero to time at which the last observable concentration was obtained was calculated by the trapezoidal method and the terminal AUC was obtained by dividing the last plasma concentration by the terminal logarithmic slope.

AUC under the concentration × *AUC* (Cp[t] < x). AUC($Cp < x$) was defined by the following equation:

$$\mathrm{AUC}(Cp < x) = \int_0^{\infty} Cp(t)\mathrm{d}t - \int_{t_1}^{t_2} (Cp(t) - x)\mathrm{d}t$$

where Cp is plasma concentration of 5-FU, t_1 and t_2 are the time when Cp equals x and $Cp(t) > x$ if $t_1 < t < t_2$, respectively.

Correlation of AUC($Cp(t) < x$) and inhibition of tumor growth was calculated by linear regression.

RESULTS

Pharmacokinetics of plasma DFUR following oral administration of DFUR without or with BVDU

Plasma DFUR concentrations were measured following oral administration of DFUR without or with BVDU (Fig. 1). The pharmacokinetic parameters of DFUR are listed in Table 1. A maximum plasma DFUR concentration was reached within 15 min and decreased rapidly during the first 2 hours. With the higher doses of DFUR (300–1000 mg/kg), DFUR disappearance from the plasma seemed to slow down during the next 4-hr period (Fig. 1). AUC increased proportionally with the given doses at least up to a dose of 500 mg/kg (Table 1). The $t_{1/2}$ of terminal phase ranged from 0.35 to 3.13 hr, depending on the dose used. Co-administration of BVDU with DFUR had no appreciable effect on the plasma concentrations

Antitumor activity of oral DFUR alone and combined with oral BVDU

As shown in Fig. 3, the oral administration of DFUR alone at 100 mg/kg did not markedly affect the growth of adenocarcinoma 755 tumors in mice (T/C = 75%). If, however, DFUR (100 mg/kg) treatment was combined with BVDU (10 mg/kg) it caused a marked reduction in tumor growth (T/C = 4%). This antitumor effect was slightly better than that obtained with DFUR alone at 500 mg/kg (T/C = 10%). Tumor growth was completely suppressed if DFUR was administered at 200 or 300 mg/kg in combination with BVDU at 10 mg/kg.

The antitumor activity of DFUR was linearly correlated with the AUC or AUC ($Cp < x$) of 5-FU. The tumor growth inhibition achieved by DFUR when analyzed in function of the AUCs and AUC($Cp < x$)'s

of 5-FU showed high correlation coefficients which increased with decreasing x values.

DISCUSSION

Peak plasma concentrations of DFUR and 5-FU were reached within 15 min after oral administration of DFUR, which indicates that DFUR is rapidly absorbed and metabolized to 5-FU. After it has been administered at higher doses, DFUR disappears from the plasma in a biphasic fashion with a long terminal half-life. Also, the plasma concentration profile of 5-FU can be characterized biexponentially. The plasma 5-FU levels did not exceed 1–2% of DFUR levels. Terminal half-life of 5-FU ranged from 3 to 7 hr which is considerably higher than that observed following 5-FU administration to mice. The long half-lives of DFUR and 5-FU at higher doses may be due to a continued absorption beyond 3 hr probably from the lower portion of the intestinal tract [14].

In the body BVDU is readily converted to BVU by pyrimidine nucleoside phosphorylases which cleave the *N*-glycosidic linkage of BVDU. The resulting BVU is known to interfere with the degradative pathway of pyrimidines (i.e. 5-FU), by blocking the action of dihydrouracil dehydrogenase [15]. It is likely, therefore, that the increased plasma 5-FU levels obtained upon oral administration of DFUR plus BVDU resulted from a decreased breakdown of 5-FU. Thus, BVDU and DFUR act as prodrugs of BVU and 5-FU, respectively. A high BVU level was maintained for at least 4 hr after oral administration of BVDU (10 mg/kg). BVU also inhibits the degradation of uracil, and hence, high plasma levels of uracil can be maintained for a long time. Uracil also prevents the degradation of 5-FU [16].

While enhancing the prolonging the plasma 5-FU levels generated by DFUR, BVDU also markedly potentiated the antitumor activity of DFUR, so that at a dose of 200 and 300 mg/kg the latter achieved even complete tumor growth suppression. The therapeutic index (LD_{50}/ED_{50}) of the combination of BVDU plus DFUR was 4.3-fold greater than that of DFUR alone (Table 4).

The major toxicity of DFUR upon oral administration is diarrhea [17], which may be due to the high phosphorylase activity in the small intestine [3–5]. As the dose of DFUR can be reduced when it is combined with BVDU, the DFUR dose may fall below its toxicity threshold. The combination of DFUR + BVDU, therefore, offers great promise from a therapeutic viewpoint, and should be further pursued for its clinical potential in cancer treatment. On the other hand, the close correlation that was found between the antitumor potency of DFUR, in the absence or presence of BVDU, and the plasma 5-FU levels generated by DFUR attest to the importance of pharmacokinetic parameters in the antitumor activity of 5-FU (prodrugs).

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