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ENHANCING EFFECT OF BROMOVINYLDEOXYURIDINE ON ANTITUMOR ACTIVITY OF 5-FLUOROURACIL AGAINST ADENOCARCINOMA 755 IN MICE

INCREASED THERAPEUTIC INDEX AND CORRELATION WITH INCREASED PLASMA 5-FLUOROURACIL LEVELS

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Abstract—A marked inhibition of the growth of solid tumor adenocarcinoma 755 was achieved by the combination of 5-fluorouracil (5-FU) with bromovinyldeoxyuridine (BVDU). The therapeutic index (LD_{50}/ED_{50}) for the combination of BVDU plus 5-FU was 8.1 and 3.9 upon intraperitoneal (i.p.) or oral (p.o.) administration, respectively. The therapeutic index of i.p. 5-FU given alone was 2.3, whereas for p.o. 5-FU given alone no therapeutic index could be established because of insufficient activity of the compound. Thus, the therapeutic index of 5-FU increased significantly when combined with BVDU.

Pharmacokinetic studies revealed that upon i.p. or p.o. 5-FU administration plasma 5-FU levels rapidly declined, but that, in the combination with BVDU, the plasma clearance of 5-FU, especially following p.o. administration, was slowed down considerably. Antitumor activity of 5-FU correlated with AUC (area under the concentration × time curve), within the plasma 5-FU concentration range from 0.02 to 0.4 μg/ml.

(*E*)-5-(2-Bromovinyl)uracil (BVU) increases the antitumor activity of 5-fluorouracil (5-FU) in the P 388 leukemia [1] and MOPC 315 plasmacytomas [2] model systems. This enhancing effect is apparently due to the inhibitory effect of BVU on dihydrothymine dehydrogenase, the rate-limiting enzyme in the catabolism of pyrimidines. (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU), which is rapidly converted to BVU *in vivo* [3], results in a similar enhancement of the antitumor effect of 5-FU in the P 388 leukemia model as observed upon the injection of BVU [1]. Combination of BVDU with 5-FU also results in an enhanced inhibition of the growth of the solid tumor adenocarcinoma 755 in BDF_1 mice [4], and, similarly, BVDU enhances the antitumor activity of tegafur (FT), which can be considered as a depot form of 5-FU, against adenocarcinoma 755 [5]. These investigations indicate that BVDU which by itself is a selective antiviral compound that holds great promise for the treatment of herpes simplex virus type 1 and varicella-zoster virus infections [6], should also be pursued for its antitumor potential, particularly in combination with 5-FU.

The present studies were undertaken (i) to determine whether BVDU potentiated the antitumor activity of 5-FU to a greater extent than its toxicity and hence achieved an increased therapeutic index, and (ii) to provide a biochemical basis for the enhancing effect of BVDU on the antitumor activity of 5-FU.

MATERIALS AND METHODS

Compounds. The origin of the compounds was as described previously [4, 5].

Animals. Groups of six male BDF_1 mice (SPF) 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°.

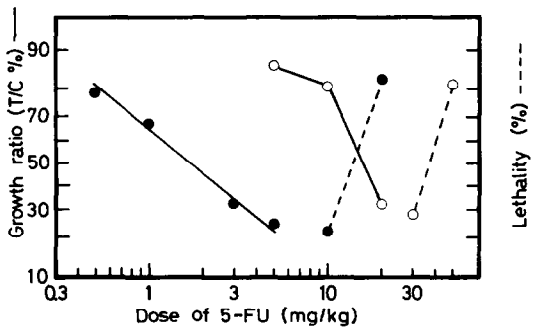

Fig. 1. Antitumor activity and toxicity of the combination 5-FU with BVDU, both administered i.p. to tumor-bearing mice. Percent growth ratio (T/C) and lethality are plotted on the ordinate. The dose of BVDU was 100 mg/kg. Each point represents mean for three experiments (six mice/group): ○, FU alone; ●, FU + BVDU.

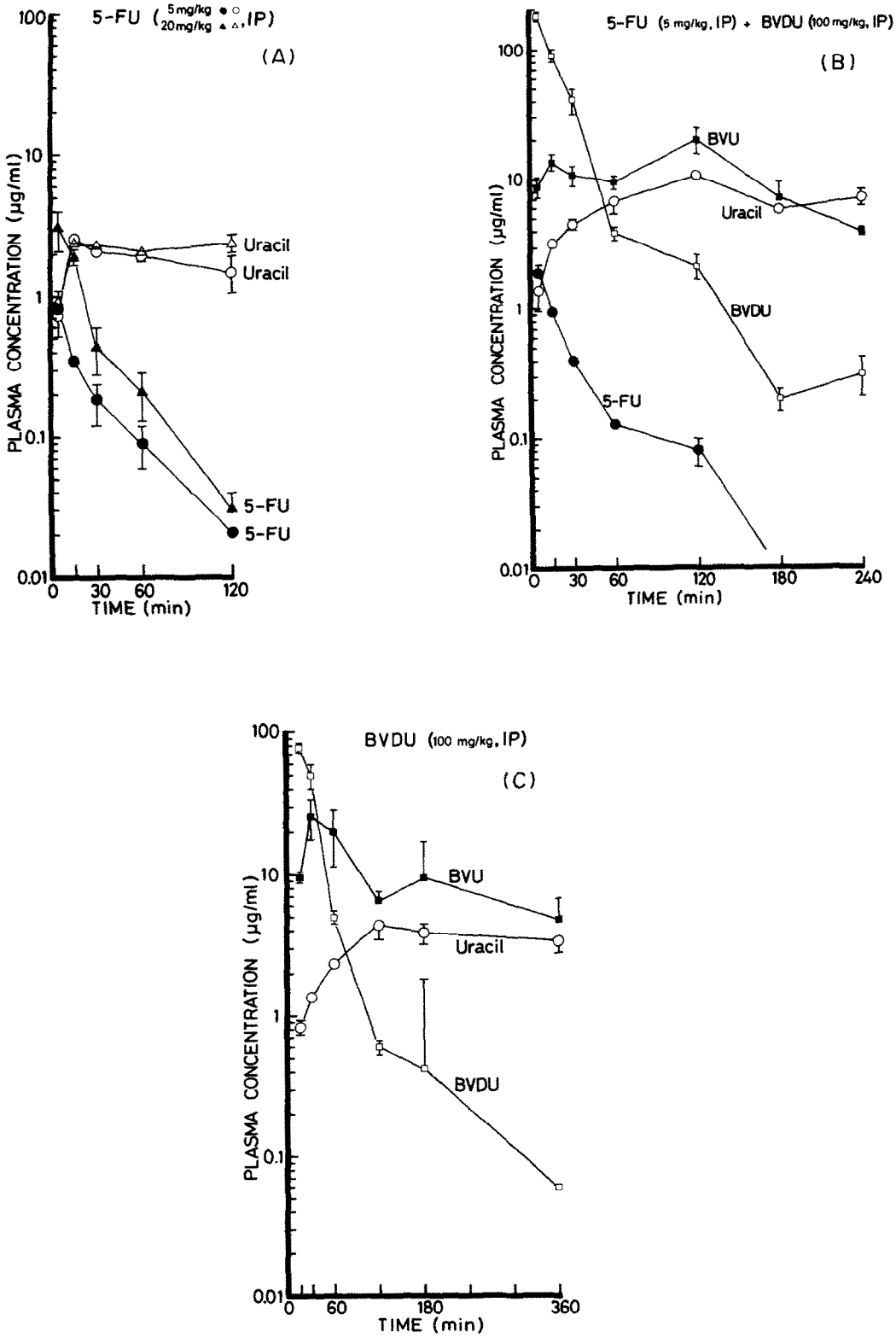

Fig. 2. Plasma levels of 5-FU, uracil, BVU and BVDU following i.p. administration of 5-FU (5 mg/kg, ○●; 20 mg/kg, △▲) (panel A), 5-FU (5 mg/kg) plus BVDU (100 mg/kg) (panel B), and BVDU (100 mg/kg) (panel C). Each point represents mean ± SE (three mice/group).

Antitumor activity and toxicity. Mice were inoculated subcutaneously on day 0 with adenocarcinoma 755 tumor cells (5×10^5 cells/mouse). The tumors had been maintained by subcutaneous 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 drugs were administered intraperitoneally (i.p.) or perorally (p.o.) daily for five consecutive days. Tumor weight was determined on day 12. 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 %). The 50% effective dose (ED_{50}) corresponded to the dose required to achieve a T/C value of 50%. In parallel with the ED_{50} determinations, 50% lethal doses (LD_{50}) were also determined.

HPLC assays. Groups of three BDF_1 mice were used. Blood samples were collected under diethyl ether anesthesia from the descending vena cava at specified times after administration of the drugs, and were immediately cooled on ice. The samples were then centrifuged for 10 min at 3000 r.p.m. at a temperature of 4°, and plasma was collected.

Plasma aliquots (0.3–0.5 ml) from individual mice were adjusted with distilled water to a total volume of 1 ml, and 0.2 ml of 0.5 M KH_2PO_4 buffer and 8 ml of ethyl acetate were added [5]. After extraction and centrifugation, the organic layer was evaporated *in vacuo* at 35°. The residue was dissolved in water, in the same volume as original, and put on Bond Elut, SAX, column (Analytichem International, Harbor City, CA), and then 25 μl of the eluted material was injected into an HPLC instrument (Toyo Soda, Tokyo, Japan) equipped with a Hibar prepacked column, LiChrosorb RP-18 (5 μm; Cica-Merck, Tokyo, Japan). 5-FU, uracil, BVU and BVDU were separated by using 2% methanol–sodium acetate buffer (10 mM, pH 4.0) for 10 min, a linear gradient from 2 to 30% methanol–sodium acetate buffer for 5 min and, finally, 30% methanol–sodium acetate buffer for 25 min. The retention times of uracil, 5-FU, BVU and BVDU were 7.0, 7.9, 30.5 and 35 min, respectively.

RESULTS

Antitumor activity and plasma 5-FU concentration following combined i.p. BVDU and 5-FU administration

Intraperitoneal administration of 5-FU alone at 5 mg/kg did not affect the growth of adenocarcinoma 755 in mice (T/C = 86%). If, however, 5-FU treatment was combined with BVDU, 5-FU at 5 mg/kg caused a marked reduction of tumor growth (T/C = 24%) (Fig. 1). Peak concentrations of 5-FU in plasma at 5 min after i.p. administration of 5-FU alone and in combination with BVDU were 0.80 ± 0.31 and 1.88 ± 0.32 μg/ml, respectively (Fig. 2). 5-FU was rapidly cleared from the plasma, and at 60 min after 5-FU administration, plasma 5-FU level was only one-tenth of the peak plasma level (Fig. 2A, B). At 120 min after 5-FU injection, the plasma 5-FU level was about 5-fold higher if 5-FU had been combined with BVDU than if it had been given alone.

The levels of BVU generated following the administration of BVDU remained high (≥5 μg/ml) during the whole observation period (up till 360 min), although BVDU itself was cleared from the plasma for 99.9% within 3 hr, whether it was given alone (Fig. 2C) or in combination with 5-FU (Fig. 2B).

Levels of uracil, which is a normal component of plasma, were also followed following i.p. administration of 5-FU and BVDU. Uracil is known to increase the antitumor activity of 5-FU [7]. If BVDU, via BVU, inhibits dihydrothymine dehydrogenase as presumed [1], plasma uracil levels may increase following BVDU administration and this proved to be the case. Plasma uracil levels raised from approximately 2 μg/ml to 7 μg/ml following i.p. administration of BVDU (Fig. 2B, C).

Antitumor activity and plasma 5-FU concentration following combined p.o. BVDU and 5-FU administration

The combination of p.o. 5-FU plus p.o. BVDU was investigated for its inhibitory effect on the growth of adenocarcinoma 755 under exactly the same conditions as used for investigating the antitumor activity of i.p. 5-FU plus i.p. BVDU. At the maximum nontoxic dose (30 mg/kg) at which 5-FU could be administered p.o., it brought about 40% reduction in tumor growth (T/C: 60%). At 5 mg/kg, p.o. 5-FU had virtually no effect on tumor growth, but if combined with BVDU (100 mg/kg), it effected a 97% reduction in tumor weight (Fig. 3).

When 5-FU was administered p.o. at 5 or 30 mg/kg, it was cleared from the plasma within 60 min (Fig. 4A). If the administration of 5-FU (5 mg/kg) had been combined with BVDU (100 mg/kg), a high 5-FU level (higher than 0.1 μg/ml) was sustained for at least 120 min, and only thereafter plasma 5-FU concentrations gradually decreased (Fig. 4B). Thus, the plasma clearance of p.o. administered 5-FU was slowed down considerably in the presence of BVDU.

Plasma BVDU level decreased more slowly after p.o. than after i.p. administration so that its clearance from the plasma was only 90–95% at 3 hr after its administration (Fig. 4B, C). High BVU levels were maintained for at least 6 hr after administration

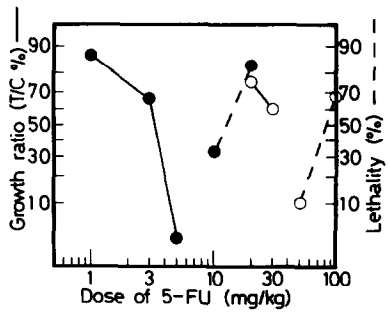

Fig. 3. Antitumor activity and toxicity of the combination 5-FU with BVDU, both administered p.o. to tumor-bearing mice. Percent growth ratio (T/C) and lethality are plotted on the ordinate. The dose of BVDU was 100 mg/kg. Each point represents mean for at least two experiments (six mice/group): ○, FU alone; ●, FU + BVDU.

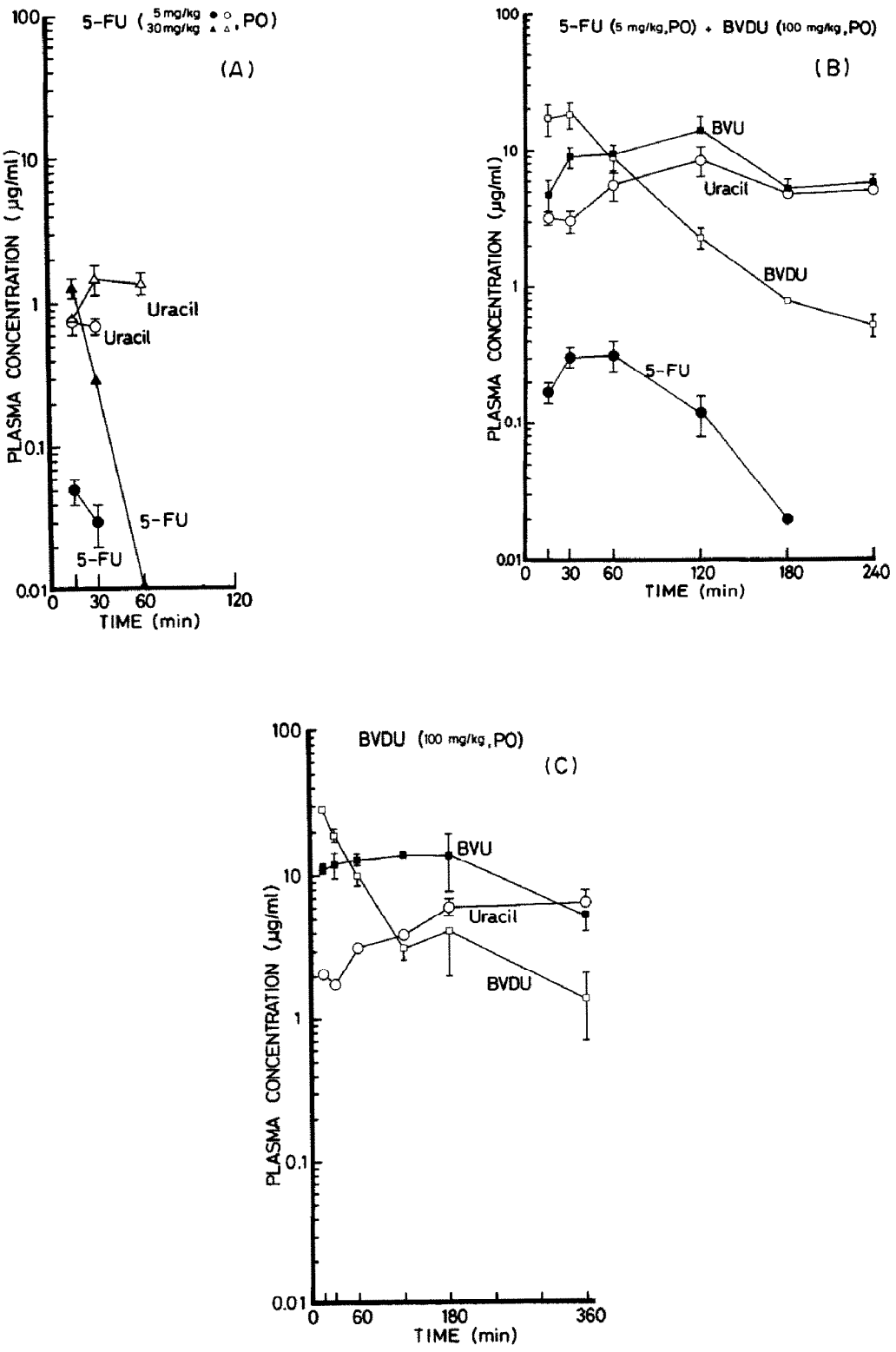

Fig. 4. Plasma levels of 5-FU, uracil, BVU and BVDU following p.o. administration of 5-FU (5 mg/kg, ○●; 30 mg/kg, △▲) (panel A), 5-FU (5 mg/kg) plus BVDU (100 mg/kg) (panel B), and BVDU (100 mg/kg) (panel C). Each point represents mean ± SE (three mice/group).

Table 1. Therapeutic index of 5-FU combined or not with BVDU

	ED_{50}* (mg/kg/day)	LD_{50}*	Therapeutic index†
I.P.			
5-FU	15.8	37.0	2.3
5-FU + BVDU (100 mg/kg)	1.7	13.8	8.1
P.O.			
5-FU	>30	82.0	—
5-FU + BVDU (100 mg/kg)	3.3	13.0	3.9

* Abbreviations for ED_{50} and LD_{50} as explained in Materials and Methods. LD_{50} was calculated from survivors on day 12 after the 5 consecutive daily administrations of the compounds on days 1–5.

† Therapeutic index: LD_{50}/ED_{50}.

of BVDU, whether the latter had been administered i.p. (Fig. 2C) or p.o. (Fig. 4C).

Plasma uracil levels were increased from approximately 0.7 μg/ml to ≥5 μg/ml following p.o. administration of BVDU (Figs. 4A–C), which corroborates the results obtained upon i.p. administration of BVDU (Fig. 2).

DISCUSSION

5-FU has been used extensively in the adjuvant chemotherapy of various human tumors. BVDU enhances the antitumor activity of 5-FU through an inhibitory effect on its degradative pathway [1]. Combination of 5-FU with BVDU not only increases the antitumor activity of 5-FU but also its toxicity for the host. However, the therapeutic index (LD_{50}/ED_{50}) of the combination of i.p. BVDU plus i.p. 5-FU is about 3.5-fold greater than that of 5-FU alone (Table 1). Following p.o. administration, the combination of BVDU with 5-FU achieves a marked inhibition of tumor growth (97% inhibition if 100 mg/kg BVDU is combined with 5 mg/kg 5-FU), although the maximum inhibition effected by 5-FU if used alone at the optimal dose is not greater than 40%. These results indicate, therefore, that BVDU enhances the antitumor potency of 5-FU to a significantly greater extent than its toxicity.

An obvious reason for the potentiating effect of BVDU on the antitumor activity of 5-FU may be an inhibition of the degradation of 5-FU [1], thus resulting in higher plasma levels of 5-FU. As shown in Fig. 5, the antitumor activity of 5-FU correlated closely with the AUC, within the plasma 5-FU concentration range from 0.02 to 0.40 μg/ml ($r = 0.97$), but not with the total AUC (plasma FU concentrations ≥ 0.02 μg/ml) ($r = 0.55$). This indicates that a continuously high plasma 5-FU concentration rather than its maximum peak level is the critical determinant of the antitumor activity of 5-FU.

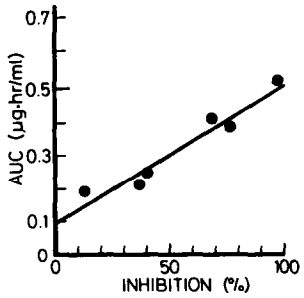

Fig. 5. Correlation of antitumor activity, expressed as inhibition ratio (100-T/C%) with plasma 5-FU levels, expressed as AUC ($0.02 \leq C \leq 0.40$); $r = 0.97$, $P < 0.01$.

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