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Enhancing Effect of Bromovinyldeoxyuridine on Antitumor Activity of 5-Fluorouracil and Ftorafur Against Adenocarcinoma 755 in Mice*

MASAAKI IIGO,† YOKO NAKAJIMA,† AKIO HOSHI† and ERIK DE CLERCQ‡§

†*Pharmacology Division, National Cancer Center Research Institute, Tsukiji 5-chome, Chuo-ku, Tokyo* 104, *Japan and* ‡*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B*-3000 *Leuven, Belgium*

Abstract—*When combined with bromovinyldeoxyuridine (BVdUrd), 5-fluorouracil (FUra) brought about a significant reduction in the growth of adenocarcinoma* 755 *tumors in mice, at doses at which either drug used alone (BVdUrd*: 100 mg/kg) *did not effect an appreciable antitumor activity. BVdUrd also increased the toxicity of FUra for the hosts but not commensurately with its enhancing effect on the antitumor activity of FUra. BVdUrd also potentiated the antitumor activity of ftorafur, so that doses of ftorafur* (50 *or* 100 mg/kg) *which by themselves did not cause a significant reduction in tumor growth became markedly effective when combined with BVdUrd at a dose as low as* 10 mg/kg. *For some combinations of BVdUrd with FUra, the antitumor potency was further enhanced by the administration of* L-*cysteine* (300 mg/kg).

INTRODUCTION

UPON systemic i.p. (intraperitoneal) administration to rats, the potent antiherpetic compound (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (bromovinyldeoxyuridine, BVdUrd) [1] is rapidly degraded by pyrimidine nucleoside phosphorylases to (*E*)-5-(2-bromovinyl)uracil (bromovinyluracil, BVUra) [2]. In contrast with BVdUrd which is completely cleared from the bloodstream within 2–3 hr, BVUra persists in the circulation for at least 24 hr. In this sense, BVUra differs from other 5-substituted uracils, e.g. 5-fluorouracil (FUra), which are cleared from the plasma within 2–3 hr [2]. Unlike other 5-substituted uracils, BVUra is not a substrate for dihydrothymine dehydrogenase, the enzyme that initiates the catabolic pathway of pyrimidine bases [3]. On the contrary, BVUra inhibits the enzyme, and by doing so, it slows down the degradation of other 5-substituted uracils, including FUra [4].

BVUra thus increases the half-life of FUra [4], and therefore may be expected to enhance the antitumor activity of FUra. In fact, BVUra was found to enhance the antitumor activity of FUra in DBA/2 mice inoculated with P388 leukemia cells, and so did BVdUrd, apparently through the release of BVUra [3]. In another murine tumor model, MOPC-315 plasmacytoma transplanted to BALB/c mice, combination of FUra (either 12.5 or 25 mg/kg) with BVdUrd (69.5 mg/kg) effected a significant reduction in tumor growth, while either drug used alone did not give any response [5].

To establish whether the synergistic antitumor action of FUra and BVdUrd may extend to other tumor systems and varying experimental conditions, combination of FUra and BVdUrd were explored against adenocarcinoma 755 in BDF_1 mice. This tumor model has been used previously to demonstrate the potentiating effect of L-cysteine and L-cystine on the antitumor activity of FUra and ftorafur (tegafur) [6, 7]. The adenocarcinoma 755 model has been adopted by the Cancer Chemotherapy National Service Center (CCNSC) of the U.S. National Cancer Institute for the screening of antitumor agents of potential clinical value [8]. Further details on the tumor doubling time and chemotherapeutic response are provided in the review article of Skipper [9]: adenocarcinoma 755 is considered curable by cytoxan followed by 6-mercaptopurine when tumors are about 0.5 g.

The adenocarcinoma 755 tumor model was also used to evaluate the antitumor activity of combinations of BVdUrd with ftorafur. Ftorafur has shown significant antitumor activity against several

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§To whom requests for reprints should be addressed.

adenocarcinomas with a potency similar to, but of lesser toxicity than, FUra [10–12]. Ftorafur is considered to be a depot form of FUra [13–16], which is generated from ftorafur *in vivo* (in the liver) with the aid of cytochrome P-450 [17, 18]. FUra is then further degraded in the liver [19] following a reductive pathway involving dihydrothymine dehydrogenase. Degradation of FUra can be inhibited by uracil [20] and this leads to an increase in FUra levels in blood and tumor tissue. Co-administration of uracil [20] or thymine [21] with ftorafur also increases the plasma and tumor levels of FUra.

MATERIALS AND METHODS

Drugs

FUra was obtained from Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan and ftorafur was provided by SS Pharmaceutical Co. Ltd., Narita, Japan. L-Cysteine was purchased from Sigma Chemical Co. (St. Louis, Mo, U.S.A.). BVdUrd was synthesized essentially as described by Jones *et al.* [22]. BVdUrd was dissolved in a 10% solution of ethanol in physiological saline; the other drugs were dissolved in 0.9% saline solution, and all drugs were administered intravenously (i.v.).

Animals

Groups of 6 male BDF_1 mice with body weight of 21–23 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were housed in plastic cages with wood chip bedding and received a CA-1 pellet diet (CLEA Japan, Inc., Tokyo, Japan) and water *ad libitum*. All experiments were performed in an animal laboratory at a controlled temperature (25°C).

Inoculation and treatment of adenocarcinoma 755

Adenocarcinoma 755 tumor cells (5×10^5 cells/mouse) were inoculated subcutaneously (s.c.) on day 0. The tumors had been maintained by s.c. transfer every 12 days in syngeneic mice in our laboratory at the National Cancer Center Research Institute, Tokyo, Japan.

Beginning 24 hr after tumor implantation, the mice were given daily i.v. injections of the compounds for 4 consecutive days. The tumor weight was determined on day 12.

Evaluation of antitumor activity and toxicity

Antitumor activity was evaluated by calculating the T/C ratio, or ratio of the average tumor weight in the treated groups to that in the control group on day 12. Data were analyzed for significance by the 2-tailed Student's *t*-test.

In addition to the tumor weight on day 12, average body weight was assessed on day 0 and day 12. The term 'toxicity' was reserved for the drug or drug combinations causing mortality.

RESULTS

FUra combined with BVdUrd and/or L-*cysteine*

When used alone, FUra was effective in reducing the growth of adenocarcinoma 755 (T/C: 12–13%: Table 1, Exp. I and II), only at a dose of 20 mg/kg; at a 1.5-fold higher dose FUra proved toxic for the host. At a dose of 5 or 10 mg/kg, FUra did not achieve a significant reduction in tumor growth. If, however, this FUra dose was combined with BVdUrd (100 mg/kg) a significant reduction in tumor growth was observed. BVdUrd also enhanced the toxicity of FUra for the host, but not commensurately with its potentiating effect on the antitumor activity of FUra. Thus, BVdUrd decreased the toxic dose of FUra from 30 to 20 mg/kg, that is 1.5-fold; but, on the other hand, BVdUrd decreased the effective antitumor dose of FUra from 20 to 5 mg/kg, that is 4-fold (Table 1, Exp. I). At 100 mg/kg, L-cysteine had no marked effect on the antitumor activity of FUra (Table 1, Exp. I); however, at 300 mg/kg, L-cysteine enhanced the antitumor activity of FUra (20 mg/kg) without increasing its toxicity for the host (Table 1, Exp. II). When both compounds, L-cysteine and BVdUrd, were combined with FUra, the antitumor activity of FUra was potentiated as compared to FUra alone or FUra combined with BVdUrd. Under the ideal conditions [FUra (10 mg/kg), BVdUrd (100 mg/kg) and L-cysteine (100 mg/kg)], tumor development was almost totally suppressed (T/C: 1%) (Table 1, Exp. I). At the dose of 300 mg/kg, L-cysteine also reduced the toxicity of FUra. Thus, the combination of FUra (20 mg/kg) + BVdUrd (100 mg/kg) was less toxic for the host if combined with L-cysteine at 300 mg/kg (Table 1, Exp. II). This is in line with previous studies indicating that L-cysteine may potentiate the antitumor activity of FUra without increasing its toxicity [6, 23].

Ftorafur combined with BVdUrd and/or L-*cysteine*

With ftorafur no significant reduction in tumor growth was achieved if used at a dose of 50 or 100 mg/kg, and at a dose of 200 mg/kg it was toxic (Table 2). If, however, ftorafur (50 or 100 mg/kg) was combined with BVdUrd (10 mg/kg), there was a dramatic reduction in tumor growth, without concomitant increase in toxicity for the host. With the combination ftorafur (100 mg/kg) + BVdUrd (10 mg/kg), virtually no tumor development was observed (Table 2, Exp. III). A dose of 3 mg/kg sufficed for BVdUrd to enhance the antitumor activity of ftorafur (50 or 100 mg/kg), and in the presence of L-cysteine the latter effect became statistically significant (Table 2, Exp. II).

DISCUSSION

BVdUrd caused a significant enhancement in the antitumor activity of FUra against adenocarcinoma

Table 1. *Inhibition of growth of adenocarcinoma* 755 *tumors in* BDF_1 *mice by FUra in combination with BVdUrd and/or* L-*cysteine*

	Body weight change Mean (g)	Tumor weight on day 12 Mean ± S.D. (mg)	T/C(%)
Exp. I.			
Control	+ 1.6	3544 ± 930	
FUra (5)*	1.4	2426 ± 564	69
FUra (10)	+ 4.1	1653 ± 493	47
FUra (20)	+ 0.3	462 ± 45	13
FUra (30)		Toxic (5/6)†	
FUra (5) + L-cysteine (100)	+ 2.7	2616 ± 687	74
FUra (10) + L-cysteine (100)	+ 3.7	1310 ± 689	37
FUra (20) + L-cysteine (100)	+ 2.0	318 ± 173	9
FUra (30) + L-cysteine (100)		Toxic (4/6)	
FUra (5) + BVdUrd (100)	+ 3.9	604 ± 307‡	17
FUra (10) + BVdUrd (100)	+ 0.1	287 ± 262‡ (1/6)	8
FUra (20) + BVdUrd (100)		Toxic (6/6)	
FUra (5) + BVdUrd (100) + L-cysteine (100)	+ 2.8	769 ± 150‡	22
FUra (10) + BVdUrd (100) + L-cysteine (100)	− 2.0	39 ± 28§‖	1
FUra (20) + BVdUrd (100) + L-cysteine (100)		Toxic (6/6)	
Exp. II.			
Control	+ 4.1	3370 ± 363	
FUra (5)	+ 3.6	2212 ± 489	66
FUra (10)	+ 4.1	1849 ± 411	55
FUra (20)	+ 1.7	419 ± 118	12
FUra (5) + L-cysteine (300)	+ 2.7	2100 ± 1126	62
FUra (10) + L-cysteine (300)	+ 3.9	1633 ± 761	48
FUra (20) + L-cysteine (300)	+ 1.0	38 ± 31§	1
FUra (5) + BVdUrd (100)	+ 2.0	1539 ± 997	46
FUra (10) + BVdUrd (100)	0	217 ± 110¶	6
FUra (20) + BVdUrd (100)		Toxic (6/6)	
FUra (5) + BVdUrd (100) + L-cysteine (300)	+ 3.4	1736 ± 647	
FUra (10) + BVdUrd (100) + L-cysteine (300)	- 1.2	151 ± 79§	4
FUra (20) + BVdUrd (100) + L-cysteine (300)	- 0.6	20 ± 4§,** (1/6)	1
L-Cysteine (300)	+ 3.3	2527 ± 519	75
BVdUrd (100)	+ 3.1	2840 ± 1065	84

Body weight change was calculated as the average body weight on day 12 minus the average body weight on day 0 minus the tumor weight on day 12.

*Numbers in parentheses: mg/kg/day.

†Numbers in parentheses: dead mice/total number of mice.

‡Different from FUra (5 or 10 mg/kg) alone: $P < 0.001$.

§Different from FUra (10 or 20 mg/kg) alone: $P < 0.001$.

‖Different from the combination of FUra (10 mg/kg) with BVdUrd (100 mg/kg): $P < 0.05$.

¶Different from FUra (10 mg/kg) alone: $P < 0.01$.

**Different from the combination of FUra (10 mg/kg) with BVdUrd (100 mg/kg): $P < 0.01$.

755, at doses BVdUrd and FUra which were non-toxic to the host, so that for certain dosage regimens an almost complete suppression of tumor growth was achieved. Similarly, BVdUrd markedly potentiated the antitumor activity of ftorafur, the most dramatic effect being obtained with the combination ftorafur (100 mg/kg) plus BVdUrd (10 mg/kg). Under certain conditions, the antitumor activity of the combinations FUra + BVdUrd and ftorafur + BVdUrd could be further enhanced by adding L-cysteine (300 mg/kg) to the combinations.

The enhancing effect of BVdUrd on the antitumor activity of FUra and ftorafur is most likely mediated by BVUra which is rapidly released from BVdUrd through the action of pyrimidine nucleoside phosphorylases [2]. BVUra has been shown previously [3] to suppress the degradative pathway of pyrimidines initiated by dihydrothymine dehydrogenase. It is postulated, therefore, that by acting as a prodrug of BVUra, BVdUrd increases the bioavailability, and thereby the antitumor activity, of FUra, whether the latter is administered as such or in the form of its precursor, ftorafur.

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Table 2. Inhibition of growth of adenocarcinoma 755 tumors in BDF_1 mice by ftorafur in combination with BVdUrd and/or L-cysteine

	Body weight change Mean (g)	Tumor weight on day 12 Mean ± S.D. (mg)	T/C(%)
Exp. I.			
Control	+ 2.6	2640 ± 716	
Ftorafur (50)*	+ 2.6	1174 ± 895	44
Ftorafur (50) + BVdUrd (30)		Toxic (6/6)†	
Ftorafur (50) + BVdUrd (10)	0	196 ± 307‡	7
Ftorafur (50) + BVdUrd (3)	+ 2.3	606 ± 498	23
Ftorafur (50) + BVdUrd (1)	+ 5.6	1149 ± 413	44
Exp. II.			
Control	+ 1.5	3615 ± 559	
Ftorafur (100)	+ 2.4	1626 ± 540	45
Ftorafur (100) + L-cysteine (300)	+ 2.7	2494 ± 436	69
Ftorafur (100) + BVdUrd (3)	+ 2.8	1018 ± 360	28
Ftorafur (100) + BVdUd (3) + L-cysteine (300)	+ 1.5	871 ± 406§	24
Exp. III.			
Control		2069 ± 405	
Ftorafur (100)	+ 2.2	1025 ± 584	50
Ftorafur (100) + L-cysteine (300)	+ 1.7	1221 ± 398	59
Ftorafur (100) + BVdUrd (10)	− 5.3	9 ± 4‖	0
Ftorafur (100) + BVdUrd (10) + L-ctsteine (300)		Toxic (5/6)	
Ftorafur (200)		Toxic (3/6)	

Body weight change was calculated as explained in the footnote to Table 1.
*Numbers in parentheses: mg/kg/day.
†Numbers in parentheses: dead mice/total number of mice.
‡Different from ftorafur (50 mg/kg) alone: $P < 0.05$.
§Different from ftorafur (100 mg/kg) alone: $P < 0.05$.
‖Different from ftorafur (100 mg/kg) alone: $P < 0.001$.

REFERENCES

1. De Clercq E, Walker RT. Synthesis and antiviral properties of 5-vinylpyrimidine nucleoside analogs. *Pharmac Ther* 1984, **26**, 1–44.
2. Desgranges C, Razaka G, Drouillet F, Bricaud H, Herdewijn P, De Clercq E. Regeneration of the antiviral drug (*E*)-(2-bromovinyl)-2′-deoxyuridine *in vivo*. *Nucleic Acids Res* 1984, **12**, 2081–2090.
3. Desgranges C, Razaka G, De Clercq E, *et al.* Effect of (*E*)-5-(2-bromovinyl)-uracil on the catabolism and antitumor activity of 5-fluorouracil in rats and leukemic mice. *Cancer Res* 1986, **46**, 1094–1101.
4. Desgranges C, Razaka G, Bricaud H, De Clercq E. Inhibition of the degradation of thymine and 5-substituted uracil analogues by (*E*)-5-(2-bromovinyl)-uracil *in vivo*. *Biochem Pharmacol* 1985, **34**, 403–404.
5. Ben-Efraim S, Shoval S, De Clercq E. Enhancing effect of bromovinyldeoxyuridine on antitumour activity of 5-fluorouracil in mice bearing MOPC-315 plasmacytoma tumours. *Brit J Cancer* (In press).
6. Iigo M, Hoshi A. Mechanism of potentiation of antitumor activity of 5-fluorouracil against adenocarcinoma 755 by L-cysteine. *Biochem Pharmacol* 1986, **35**, 727–730.
7. Iigo M, Hoshi A, Kitagawa H. Potentiation of the chemotherapeutic action of tegafur against solid adenocarcinoma 755 by combination with L-cystine. *J Cancer Res Clin Oncol* 1986, **111**, 187–190.
8. Johnson RK, Goldin A. The clinical impact of screening and other experimental tumor studies. *Cancer Treat Rev* 1975, **2**, 1–31.
9. Skipper HE. The cell cycle and chemotherapy of cancer. In: Baserga R, ed. *The Cell Cycle and Cancer*. New York, Marcel Dekker, 1971, 358–387.
10. Blokhina NG, Vozny EK, Garin AM. Results of treatment of malignant tumors with ftorafur. *Cancer* 1972, **30**, 390–392.
11. Karev NI, Blokhina NG, Vozny EK, Pershin MP. Experience with ftorafur treatment in breast cancer. *Neoplasma* 1972, **19**, 347–350.
12. Valdivieso M, Bodey GP, Gottlieb JA, Freireich EJ. Clinical evaluation of ftorafur (pyrimidine-deoxyribose N_1-2′-furanidyl-5-fluorouracil). *Cancer Res* 1976, **36**, 1821–1824.

13. Cohen AM. The disposition of ftorafur in rats after intravenous administration. *Drug Metab Disposition* 1975, **3**, 303–308.
14. Fujii S, Okuda H, Akazawa A, *et al.* Studies on the fate of L-(2-tetrahydrofuryl)-5-fluorouracil (FT-207), a carcinostatic agent. III. Absorption, distribution, excretion and metabolism after rectal administration of FT-207. *J Pharm Soc Japan* 1975, **95**, 732–740.
15. Fujimoto S, Akao T, Itoh B, *et al.* Effect of N_1-(2′-tetrahydrofuryl)-5-fluorouracil and 5-fluorouracil on nucleic acid and protein biosyntheses in Ehrlich ascites cells. *Cancer Res* 1976, **36**, 33–36.
16. Smolyanskaya AZ, Tugarinov OA. The biological activity of antitumor antimetabolite ftorafur. *Neoplasma* 1972, **19**, 341–345.
17. Fujita H, Sugiyama M, Kimura K. Phamacokinetics of ftorafur (FT-207) for a clinical application. In: Hellman K, Conners TA, eds. *Cancer Chemotherapy* II (*Proceedings of the Ninth International Congress of Chemotherapy, London*, 1975). New York, Plenum Press, 1976, Vol. 8, 51–57.
18. Ohira S, Maezawa S, Watanabe K, Kitada K, Saito T. Experimental approach to increase the effect of cancer chemotherapy in tumor-bearing rats pretreated with an inducer on microsomal drug-metabolizing enzyme (cytochrome P-450). In: Hellman K, Conners TA, eds. *Cancer Chemotherapy* II (*Proceedings of the Ninth International Congress of Chemotherapy, London*, 1975). New York, Plenum Press, 1976, Vol. 8, 197–202.
19. Chaudhuri NK, Mukherjee KL, Heidelberger C. Studies on fluorinated pyrimidines VII. The degradative pathway. *Biochem Pharmacol* 1958, **1**, 328–341.
20. Fujii S, Ikenaka K, Fukushima M, Shirasaka T. Effect of uracil and its derivatives on antitumor activity of 5-fluorouracil and 1-(2-tetrahydrofuryl)-5-fluorouracil. *Gann* 1978, **69**, 763–772.
21. Fujii S, Kitano S, Ikenaka K, *et al.* Effect on co-administration of thymine or thymidine on the antitumor activity of 1-(2-tetrahydrofuryl)-5-fluorouracil and 5-fluorouracil. *Gann* 1980, **71**, 100–106.
22. Jones AS, Verhelst G, Walker RT. The synthesis of the potent anti-herpes virus agent, *E*-5(2-bromovinyl)-2′-deoxyuridine and related compounds. *Tetrahedron Lett* 1979, 4415–4418.
23. Danysz A, Wierzba K, Wutkiewicz M, Pniewska A, Praglowski T, Burba-Anczewska I. Influence of some sulfhydryl compounds on the antineoplastic effectiveness of 5-fluorouracil and 6-mercaptopurine. *Arch Immun Ther Exp* 1984, **32**, 345–349.