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Effect of (E)-5-(2-bromovinyl)-2′-deoxyuridine on Life-span and 5-fluorouracil Metabolism in Mice with Hepatic Metastases

Masaaki Iigo, Ken-ichi Nishikata, Yoko Nakajima, Akio Hoshi and Erik De Clercq

5-Fluorouracil (5FU) is rapidly metabolised in the liver by dihydrouracil dehydrogenase. Bromovinyluracil is formed in the liver from (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) by pyrimidine nucleoside phosphorylase and is a potent inhibitor of dihydrouracil dehydrogenase. The co-administration of 5FU (intravenously) and BVDU (orally) was investigated in normal BDF_1 mice and in those bearing liver metastases of Lewis lung carcinoma. 5FU alone rapidly disappeared from plasma and liver within 60 min of dosing. Administered with BVDU, 5FU persisted in plasma and liver for 60–180 min. The combination also significantly enhanced the life-span of tumour-bearing mice. 5FU plus BVDU may have therapeutic potential in the treatment of primary and secondary liver tumours.
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INTRODUCTION

THE LIVER is the main site of 5-fluorouracil (5FU) metabolism [1]. In man 5FU is more extensively catabolised when administered orally than when given intravenously [2], and plasma half-life is 10–20 min [3]. The rate-limiting liver enzyme is dihydrouracil dehydrogenase which converts 5FU to 5,6-dihydro-5-FU [4]. Rapid catabolism of 5FU means that only a small fraction is converted to the active 5-fluorouridine-5′-triphosphate and 5-fluorodeoxyuridine-5′-monophosphate.

(*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) inhibits the catabolism of 5FU and increases its antitumour activity [5, 6]. BVDU is easily converted in the liver to (*E*)-5-(2-bromovinyl)uracil (BVU) by pyrimidine nucleoside phosphorylase(s) [7]. BVU is a potent inhibitor of dihydrouracil dehydrogenase [8] and enhances the antitumour activity of 5FU (and precursors thereof) in several tumour systems in mice [8–11].

The combination of BVDU with 5FU might be particularly effective against liver tumours. Therefore we examined this combination in mice bearing liver metastases.

MATERIALS AND METHODS

Chemicals

[6-^{3}H]5FU (710 GBq/mmol) was purchased from New England Nuclear. After supplemention with cold carrier, the specific activity for injection was 4.6 MBq/mg. 5FU, 5-fluorouridine, 5-fluoro-2′-deoxyuridine, 5-fluorouridine monophosphate and 5,6-dihydrouracil were purchased from Sigma. α-fluoro-β-ureidopropionic acid and α-fluoro-β-alanine were obtained from Mitsui Pharmaceuticals, Tokyo [12]. *o*-phthalaldehyde and *p*-dimethylaminobenzaldehyde were purchased from Funakoshi and Iwai Kagaku, respectively.

Correspondence to M. Iigo.
M. Iigo, K. Nishikata, Y. Nakajima and A. Hoshi are at the Chemotherapy Division, National Cancer Center Research Institute, Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan and E. De Clercq is at the Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium.

Table 1. Effect of BVDU on antitumour activity of 5FU in mice bearing liver metastases

Treatment*	Life-span (days)	% increase†	Survivors‡
Experiment I			
Control	15.7 (1.4)		0/12
BVDU (3 × 10)	17.2 (1.2)	10	0/6
5FU (20)	20.6 (1.7)	31	1/6
5FU (5)	19.2 (1.4)	22	0/6
BVDU (3 × 10) + 5FU (5)	24.6 (0.4)§	57	1/6
Experiment II			
Control	14.2 (1.2)		0/6
5FU (10)	17.3 (0.6)	22	0/6
BVDU (3 × 10) + 5FU (10)	29.0 (3.9)‖	104	2/6
BVDU (3 × 30) + 5FU (5)	23.2 (1.3)‖	63	0/6
Experiment III			
Control	19.7 (1.6)		0/7
5FU (20)	19.2 (1.1)	−3	0/6
BVDU (3 × 10) + 5FU (10)	25.8 (0.7)§	31	0/6

Mean (S.E.).
*Experiments I and II = 4th and III = 7th day after tumour cell inoculation. All doses in mg/kg per day.
†In life-span (cured mice were excluded from calculations).
‡Ratio of 60-day survivors to total number of mice.
Significant differences from: §5FU alone (20 mg/kg per day), $P < 0.01$; and ‖(10 mg/kg per day), $P < 0.01$ by two-sided Student's *t*-test.

Animals

Inbred C57BL/6 and BDF_1 mice (5 weeks old) were purchased from Japan SLC (Hamamatsu). The mice were maintained under specific pathogen-free conditions.

Induction of liver metastases

Lewis lung carcinoma was maintained subcutaneously in C57BL/6 mice. The tumour was rapidly removed, minced in

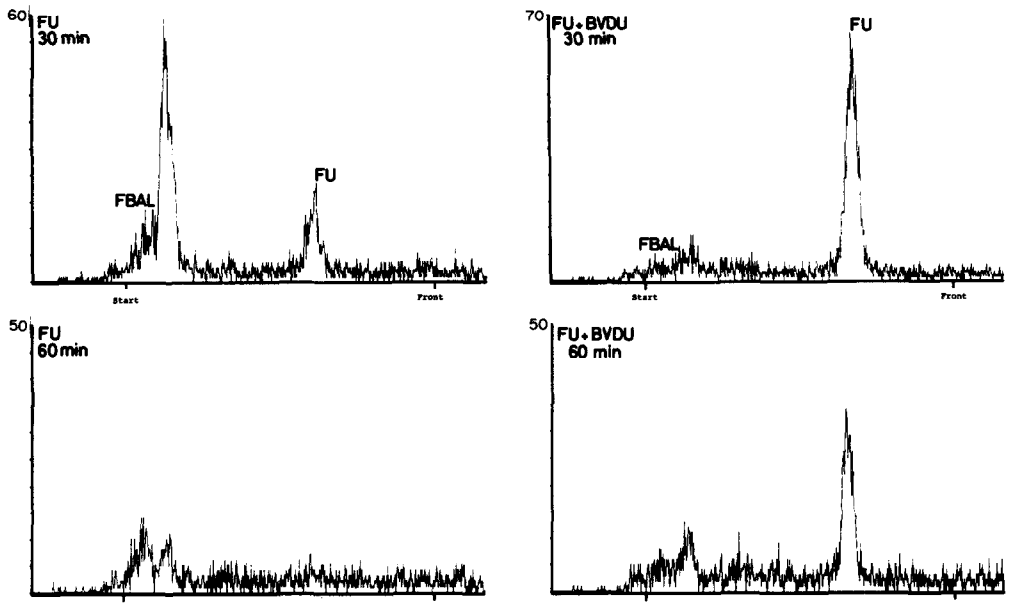

Fig. 1. Radiochromatograms of 5FU metabolites in plasma of normal mice. FBAL = α-fluoro-β-alanine.

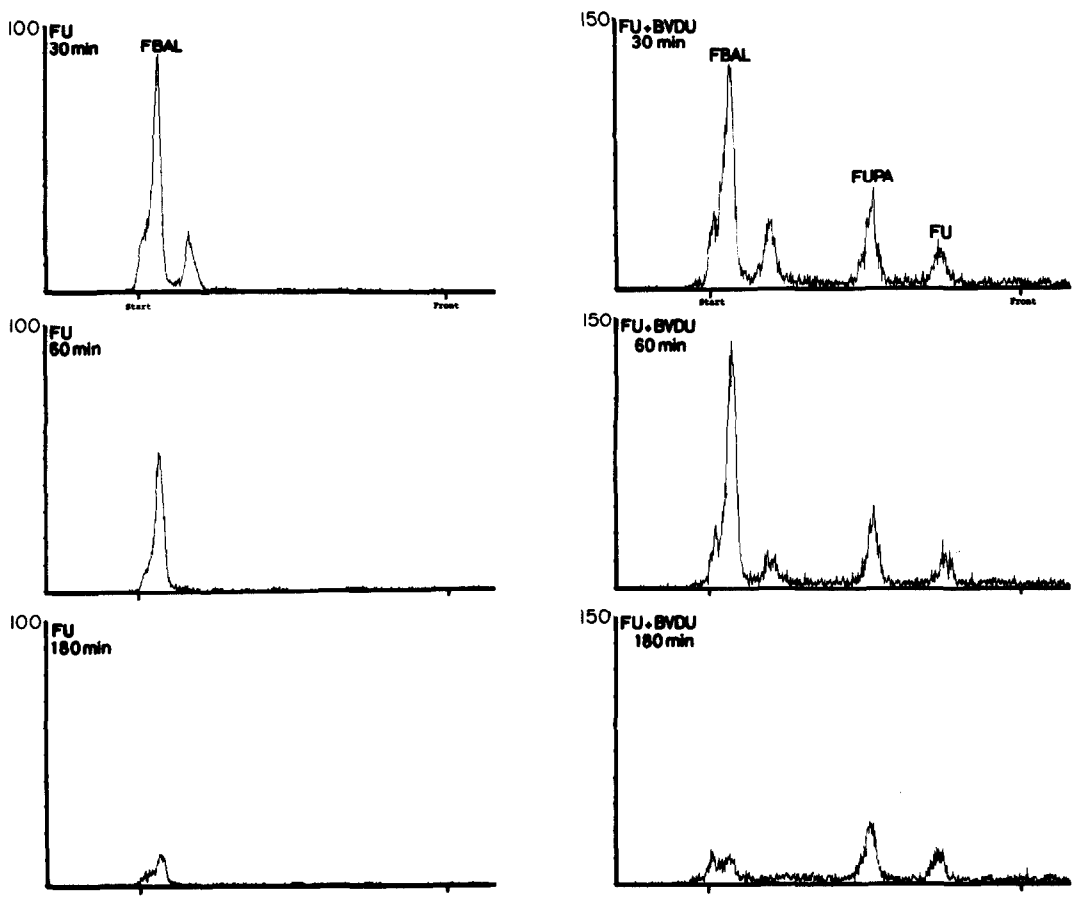

Fig. 2. Radiochromatograms of 5FU metabolites in liver of normal mice. FUPA = fluoroureidopropionic acid.

5 ml cell culture medium containing 0.3% fetal calf serum (Cedarlane, Hornby, Ontario), pressed through 120 wire mesh, washed twice with saline and adjusted to 2×10^6 cells per ml. Multiple hepatic metastases were produced in BDF_1 mice according to the method of Kopper *et al.* [13]. The mice were randomised before being distributed to cages.

Treatment, sampling and analysis

For survival analysis 5FU was administered intravenously daily for 4 consecutive days starting from the 4th or 7th day after tumour cell inoculation. BVDU was administered orally three times per day every 3 h for 4 consecutive days, also starting from the 4th or 7th day (drug doses in Table 1). The third

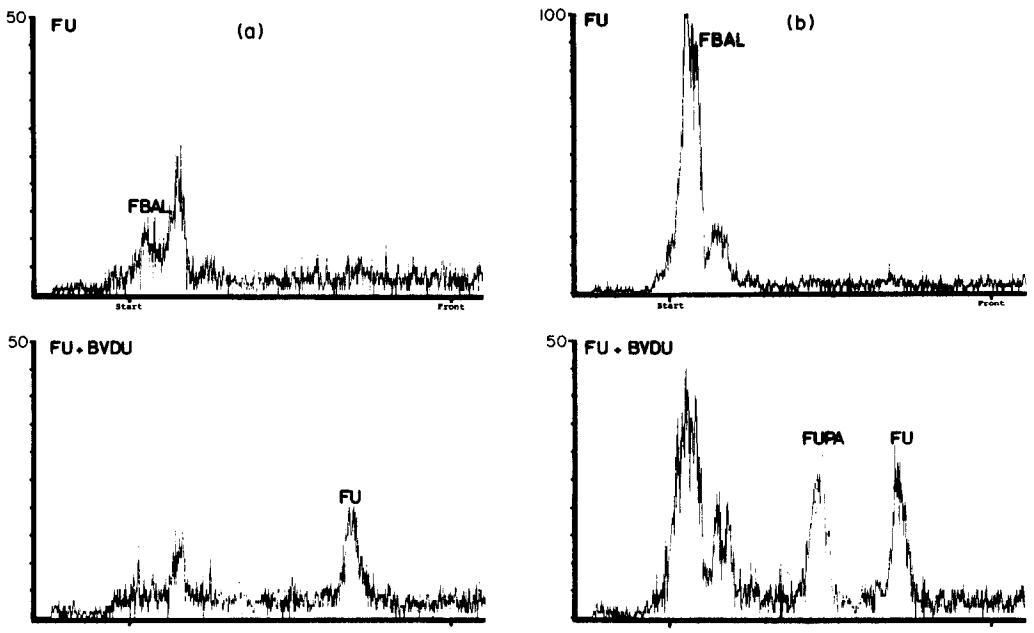

Fig. 3. Radiochromatograms of 5FU metabolites in plasma (a) and liver (b) of mice bearing hepatic metastases at 12 days after tumour cell inoculation. Plasma and liver samples were collected 60 min after [6-^{3}H]5FU administration.

daily administration of BVDU coincided with the single daily administration of 5FU. The antitumour effect was evaluated by determining the increase in life-span. At death, the mice were examined for liver metastases.

Controls and mice bearing liver metastases were studied on day 12 after tumour cell inoculation for metabolite analysis. The mice were given a single intravenous injection of [6-^{3}H]5FU (20 mg/kg; 1.9 MBq per mouse) alone or in combination with a single oral dose administration of BVDU (10 mg/kg). Mice were killed 30, 60 and 180 min later under ether anaesthesia by exsanguination from the descending vena cava. Blood was aspirated into syringes that had been wetted with heparin. Blood cells were removed by centrifugation. Promptly after the mice had been exsanguinated, the liver was removed, weighed and stored on ice. The liver was homogenised ("Polytron") in two volumes of distilled water. Plasma or liver was mixed with an equal volume of HCl/ethanol 1/100. Denatured proteins were removed by centrifugation, and 50 μl of supernatant and authentic samples were chromatographed on silica-gel plates containing a fluorescent indicator (Merck) to separate 5FU metabolites. The plates were developed with chloroform/methanol/acetic acid 30/10/5.

5FU, 5-fluorouridine and its monophosphate and 5-fluoro-2-deoxyuridine were located under ultraviolet light. 5,6-dihydrouracil was detected by colour reaction after spraying with 1 mol/l NaOH followed by acidified 1% *p*-dimethylamino-benzaldehyde (Ehrlich's reagent). α-fluoroureidopropionic acid and α-fluoro-β-alanine were detected with Ehrlich's reagent and 0.1% *o*-phthalaldehyde (0.1% 2-mercaptoethanolacetone) [14], respectively. The plates were scanned (TLC linear analyses [Berthold]) to locate radioactivity. The presence of metabolites in each radioactive zone was confirmed by autoradiography.

RESULTS

5FU metabolism in normal mice

In plasma at 30 min after administration of [6-^{3}H]5FU, 5FU and its degradation products, including α-fluoro-β-alanine, were detected. No 5FU was detected at 60 min (Fig. 1). However, if [6-^{3}H]5FU had been administered in combination with BVDU, high plasma levels of 5FU were detected 60 min later (Fig. 1).

In the liver 5FU was rapidly metabolised, the main metabolite being α-fluoro-β-alanine (Fig. 2). Even at 30 min, 5FU was no longer detected in the liver. If, however, labelled 5FU was administered with BVDU, 5FU could be found in the liver for at least 180 min (Fig. 2). Substantial amounts of α-fluoro-β-ureidopropionic acid were also detected after the combination treatment.

5FU and metabolites in mice bearing liver metastases

At 60 min after administration of [6-^{3}H]5FU, only its degradation product α-fluoro-β-alanine was detected in the plasma and liver (Fig. 3). If, however, labelled 5FU had been administered with BVDU, substantial amounts of 5FU were detected in plasma and liver 60 min later (Fig. 3).

Combined treatment and survival

Under light microscopy, liver metastases were recognised on the 7th day after tumour cell inoculation (Fig. 4). If untreated, all the mice died within 14–25 days with many metastases in the liver. Mice treated with either BVDU or 5FU showed only a slight increase in life-span (Table 1). If BVDU and 5FU were combined, life-span was increased significantly (Table 1).

The antitumour effect achieved with BVDU (3 × 10 mg/kg per day) plus 5FU (10 mg/kg per day) was greater than that observed with 3 × 30 mg/kg per day and 5 mg/kg per day, respectively. This combination in turn had slightly greater antitumour activity than a similar combination in which the dose of BVDU was reduced three-fold. At the doses used, BVDU was not toxic.

DISCUSSION

5FU was rapidly metabolised in the plasma and liver of normal mice. However, co-administration of BVDU prolonged the appearance of 5FU in plasma and liver. The same effect occurred

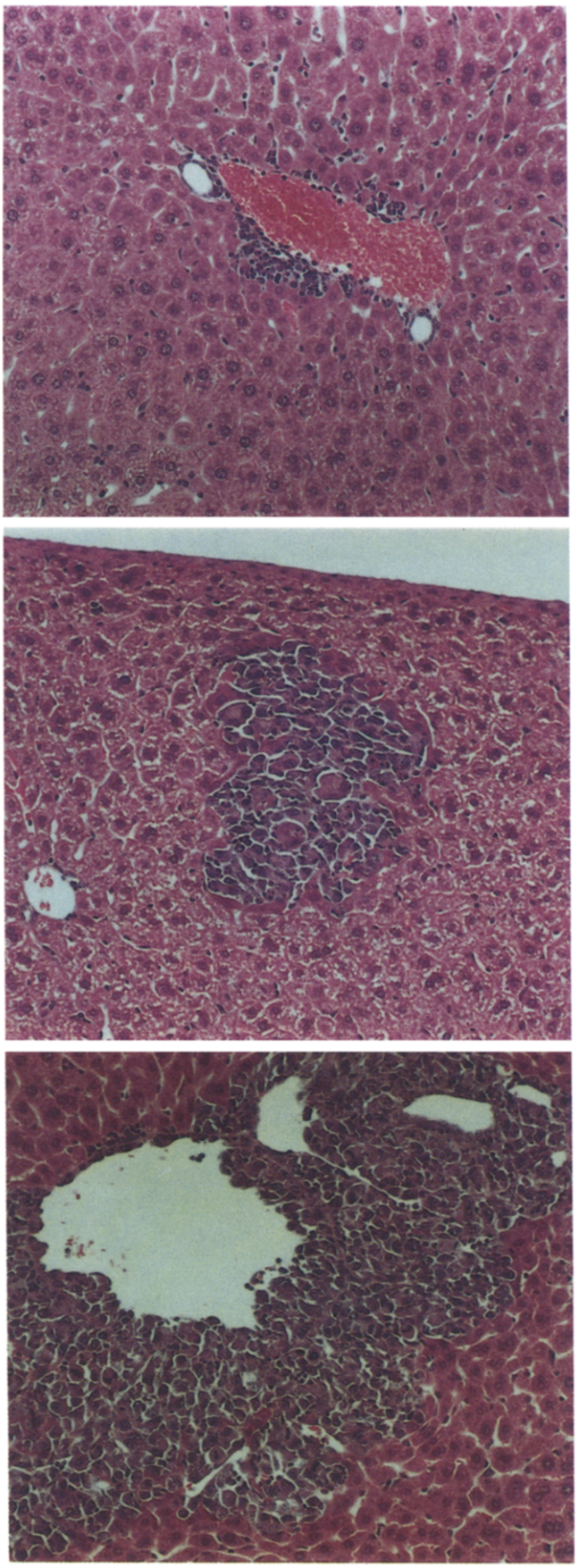

Fig. 4. Liver sections from mice at 4 (upper), 7 (middle) or 10 (lower) days after intrasplenic injection of Lewis lung carcinoma. Magnification × (170).

in mice bearing liver metastases of Lewis lung carcinoma. BVDU or its active form, (E)-5-(2-bromovinyl)uracil, inhibits dihydrouracil dehydrogenase, which degrades 5FU to 5,6-dihydro-5FU [5, 6, 8]. Moreover, higher levels of α-fluoro-β-ureidopropionic acid were detected after treatment with 5FU plus BVDU than after 5FU alone, which suggests that BVDU [or (E)-5-(2-bromovinyl)uracil] may inhibit β-ureidopropionase.

Oral administration of BVDU at 10 mg/kg three times per day is a convenient schedule, and significantly enhanced the antitumour effect of 5FU (increased life-span of mice bearing hepatic metastases). In previous experiments BVDU was non-toxic when administered intraperitoneally at 100 mg/kg per day for 5 days to tumour-bearing mice [10]. This points to the therapeutic potential of the combination of BVDU with 5FU in the treatment of both primary liver and secondary liver tumours.

1. Sommadossi J-P, Gewirtz DA, Diasio PB, Aubert C, Cano J-P, Goldman ID. Rapid catabolism of 5-fluorouracil in freshly isolated rat hepatocytes as analyzed by high performance liquid chromatography. *J Biol Chem* 1982, **257**, 8171–8176.
2. Chaudhuri NK, Mukherjee KL, Heidelberger C. Studies on fluorinated pyrimidines VII—The degradative pathway. *Biochem Pharmacol* 1958, **1**, 328–341.
3. Collins JM, Dedrick RL, King FG, Speyer JL, Myers CE. Nonlinear pharmacokinetic models for 5-fluorouracil in man: intravenous and intraperitoneal routes. *Clin Pharmacol Ther* 1980, **28**, 235–246.
4. Naguib FNM, el Kouni MH, Cha S. Enzymes of uracil catabolism in normal and neoplastic human tissues. *Cancer Res* 1985, **45**, 5405–5412.
5. Iigo M, Araki E, Nakajima Y, Hoshi A, De Clercq E. 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. *Biochem Pharmacol* 1988, **37**, 1609–1613.
6. Iigo M, Nishikata K, Nakajima Y, *et al.* 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. *Biochem Pharmacol* 1989, **38**, 1885–1889.
7. Desgranges C, Razaka G, Drouillet F, Bricaud H, Herdewijn P, De Clercq E. Regeneration of the antiviral drug (*E*)-5-(2-bromovinyl)-2'-deoxyuridine *in vivo*. *Nucleic Acids Res* 1984, **12**, 2081–2090.
8. Desgranges C, Razaka G, De Clercq E, Herdewijn P, Balzarini J, Drouillet F, Bricaud H. 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.
9. Ben-Efraim S, Shoval S, De Clercq E. Enhancing effect of bromovinyldeoxyuridine on antitumour activity of 5-fluorouracil in mice bearing MOPC-315 plasmacytomas. *Br J Cancer* 1986, **54**, 847–851.
10. Iigo M, Nakajima Y, Hoshi A, De Clercq E. Enhancing effect of bromovinyldeoxyuridine on antitumor activity of 5-fluorouracil and ftorafur against adenocarcinoma 755 in mice. *Eur J Cancer Clin Oncol* 1987, **23**, 773–777.
11. Iigo M, Yamaizumi Z, Nishimura S, Hoshi A, De Clercq E. The antitumor potency of oral tegafur against adenocarcinoma 755 in mice is markedly enhanced by oral (*E*)-5-(2-bromovinyl)-2'-deoxyuridine. *Jpn J Cancer Res* 1987, **78**, 409–413.
12. Iigo M, Nakamura A, Kuretani K, Hoshi A. Metabolic fate of 1-hexylcarbamoyl-5-fluoro-uracil after oral administration in mice. *Xenobiotica* 1980, **10**, 847–854.
13. Kopper L, Hanh TV, Lapis K. Experimental model for liver metastasis formation using Lewis lung tumor. *J Cancer Res Clin Oncol* 1982, **103**, 31–38.
14. Gunnar E, Lindeberg G. Use of *o*-phthalaldehyde for detection of amino acids and peptides on thinlayer chromatograms. *J Chromatogr* 1976, **117**, 439–441.

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