Cancer Chemother Pharmacol (1991) 29: 122–126

Cancer Chemotherapy and Pharmacology

Antitumor activity of FTC-092, a masked 5-trifluoromethyl-2′-deoxyuridine derivative

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Received 4 January 1991/Accepted 4 June 1991

Summary. 1-(3-*O*-Benzyl-2-deoxy-β-D-ribofuranosyl)-5-trifluoromethyl-2,4(1H,3H)-pyrimidinedione (FTC-092), a fluorinated pyrimidine derivative, appeared to be effective against various transplantable tumors in mice following oral administration, and its activity was superior to that of several other antitumor fluorinated pyrimidines. The ED_{50} value for FTC-092 the dose effective in achieving 50% inhibition of tumor growth against the solid form of sarcoma 180 was 13.3 mg/kg daily, whereas those for 5-trifluoromethyl-2′-deoxyuridine (CF_3dUrd), the parent compound of FTC-092, for 1-(2-tetrahydrofuryl)-5-fluorouracil (Tegafur, FT), the prodrug of 5-fluorouracil (FUra), and for FUra were 64.1, 122, and 28 mg/kg daily, respectively. The therapeutic indices (LD_{10}/ED_{50}) of FTC-092, CF_3dUrd, FT, and FUra were 4.39, 1.7, 1.35, and 1.65, respectively. FTC-092 itself is not an active agent. After it has been absorbed from the gastrointestinal tract, FTC-092 undergoes a gradual biotransformation, mainly via the action of liver microsomes, releasing CF_3dUrd over a long period. The levels of CF_3dUrd in the stomach and small intestine of mice after the oral administration of FTC-092 were undetectable, whereas those following the administration of CF_3dUrd at the same dose were high for a period of several hours. In contrast, the CF_3dUrd level generated in plasma after the administration of FTC-092 remained at a high level for a longer period than did that observed on the administration of CF_3dUrd. The low levels of CF_3dUrd measured in stomach and small-intestine tissues and the maintenance of CF_3dUrd in blood over long periods after the administration of FTC-092 are features that favor the possible clinical application of FTC-092.

Abbreviations used: FTC-092, 1-(3-*O*-benzyl-2-deoxy-β-D-ribofuranosyl)-5-trifluoromethyl-2,4(1H,3H)-pyrimidinedione; CF_3dUrd, 5-trifluoromethyl-2′-deoxyuridine; FT, 1-(2-tetrahydrofuryl)-5-fluorouracil; FUra, 5-fluorouracil; F_3Thy, 5-trifluorothymine; ILS, increase in life span

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Introduction

In previous reports [32–34], we have discussed the antitumor potency of various newly synthesized *O*-acyl, *N*-acyl, *O*-alkoxyalkyl, and *O*-alkyl derivatives of 5-trifluoromethyl-2′-deoxyuridine (CF_3dUrd). Among the CF_3dUrd derivatives investigated, FTC-092 [1-(3-*O*-benzyl-2-deoxy-β-D-ribofuranosyl)-5-trifluoromethyl-2,4-(1H,3H)-pyrimidinedione, 3′-*O*-benzyl-CF_3dUrd] was found to be the most active when given orally. CF_3dUrd was synthesized by Heidelberger et al. [15] as a thymidine analog. CF_3dUrd is known to inhibit thymidylate synthase, to interfere with DNA metabolism [11, 22, 30], and to exhibit antitumor activity against various cancer cells in culture [29] and against some transplanted tumors in mice [13]. However, the half-life of CF_3dUrd in patients' plasma is generally very short [8] due to its rapid conversion into 5-trifluorothymine (F_3Thy), an inactive agent, by thymidine phosphorylase [20]. As a result, the clinical effect of CF_3dUrd was unsatisfactory [1]. In an attempt to overcome this difficulty, we synthesized many CF_3dUrd derivatives.

In the present study, the antitumor activity of FTC-092 following its oral administration to mice bearing sarcoma 180 or L1210 leukemia were compared with those of the parent compound CF_3dUrd, of 5-fluorouracil (FUra) and of a FUra derivative, 1-(2-tetrahydrofuryl)-5-fluorouracil (Tegafur, FT). FUra was first synthesized in 1957 [10] and has been used extensively in the treatment of certain types of cancer [5, 14, 24, 25]. It inhibits thymidylate synthase and interferes with the metabolism of RNA and DNA [2, 4, 6, 7, 9, 12, 18, 19, 21, 23, 26–28, 31]. Considerable efforts have been made to find FUra derivatives that exhibit better antitumor activity and lower toxicity. Among the FUra derivatives, FT has been the subject of considerable interest in experimental and clinical cancer chemotherapy [3]. This report describes the antitumor activity of FTC-092 and discusses the reason for its improved effectiveness.

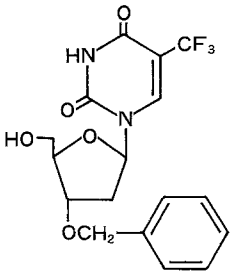

Fig. 1. Chemical structure of FTC-092

Materials and methods

Reagents. FTC-092 (Fig. 1) and FT were synthesized in our laboratory. [2-^{14}C]-FTC-092 (sp. act., 9.23 mCi/mmol) was supplied by Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). CF_3dUrd, F_3Thy and FUra were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). All other chemicals were commercial products.

Evaluation of antitumor activity. Sarcoma 180 (S180) and L1210 leukemia (L1210) were used as experimental tumor models. S180 (5×10^6 cells) was implanted s.c. into 5-week-old male ICR mice (Japan Clea Inc., Tokyo, Japan), and L1210 (1×10^5 cells) was implanted i.p. into 7-week-old male CDF_1 mice (Shizuoka Laboratory Animal Agricultural Coop., Shizuoka, Japan). A group of seven mice was used for each dose level. The compounds tested were suspended in 0.5% carboxymethyl cellulose solution containing 0.1% Tween-80 and were given p.o. at a volume of 0.1 ml/10 g mouse body weight.

The percentage of inhibition of the tumor growth of S180 was calculated by comparing the average tumor weight of drug-treated and control animals (vehicle only) on day 12. The therapeutic index (LD_{10}/ED_{50}), i.e., the relationship between the antitumor effect and the toxicity of each drug, was determined, LD_{10} being the dose causing the death of 10% of the animals and ED_{50} being the dose effective in producing 50% inhibition of the tumor growth. The percentage of increase in life span (ILS %) for animals bearing L1210 was calculated from the mean survival of the treated group as compared with that of the control group. The therapeutic ratio was expressed as ILS_{max}/ILS_{30}, where ILS_{max} is the dose achieving the maximal ILS and ILS_{30} is the dose producing 30% ILS.

Cell culture. HeLa cells were grown in 6-cm petri dishes as monolayers in Eagle's minimum essential medium containing 10% calf serum. The dishes were placed in a CO_2 incubator at 37° C for 7 days. Cells that had been harvested in their exponential phase of growth were seeded in the culture medium in each plate at a density of 1×10^4 cells/4 ml on day 0. On day 1, various concentrations of the compounds were added to the cells in the dishes. The cell number was counted on day 7 following a 5-min incubation with 0.25% trypsin to disaggregate the cells.

Determination of the rate of chemical hydrolysis of FTC-092. At a final concentration of 1×10^{-4} M, the drug solution was incubated at 37° C under various pH conditions. FTC-092 and its hydrolysis products were identified and measured by HPLC using an LC-3A liquid chromatograph (Shimazu, Japan) equipped with a UV detector operating at 254 nm. For HPLC, samples (10 µl) were applied to a column of µ-Bondapak C_{18} (Waters Associates; 300×4 mm inside diameter). The mobile phase consisted of 20 mM KH_2PO_4 and a linear gradient of ethanol (4%/min, ranging from 0 to 100%) and was run at a flow rate of 1.5 ml/min.

Extraction and determination of CF_3dUrd from plasma and various tissues. Drugs were given orally at 30 mg/kg to male ICR/JCL mice that had been starved overnight. At indicated intervals, tissue and blood samples were collected. The tissue samples were homogenized with 3 vol. saline, and the homogenates were centrifuged at 9,000 *g* for 10 min. The plasma samples and tissue supernatants (500 µl each) were deproteinized by the addition of 0.1 *N* HCl/MeOH (500 µl each) and the mixtures were centrifuged. Supernatants (10 µl) thus obtained were subjected to HPLC analysis to determine the CF_3dUrd concentration.

Assay for enzymic demasking of FTC-092. The hydrolytic activity of tissue enzymes was estimated by measuring the amount of [2-^{14}C]-CF_3dUrd that was released from [2-^{14}C]-FTC-092. Tissue samples (1 g) from male ICR/JCL mice were minced with scissors and homogenized in an ultrasonic homogenizer with 3 vol. 50 mM TRIS-HCl buffer (pH 7.4) containing 25 mM KCl, 5 mM $MgCl_2$ and 0.1 mM ethylenediaminetetraacetic acid (EDTA). The homogenates were centrifuged at 9,000 g for 30 min at 4° C, and the supernatants were used to determine the rate and extent of the hydrolysis of FTC-092. The hydrolytic activity in the serum was also determined. Tumor tissues were collected from mice on day 10 after s.c. inoculation.

The reaction mixture consisted of 10 µM [2-^{14}C]-FTC-092 (sp. act., 9.23 mCi/mmol), 50 mM TRIS-HCl buffer (pH 7.4), 25 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), and 20% tissue homogenates in a final volume of 0.5 ml. After incubation at 37° C for 60 min, the reaction was terminated by the addition of 0.1 *N* HCl/MeOH, and the [2-^{14}C]-CF_3dUrd released was quantitated using thin-layer chromatography (TLC) on silica gel (60 F_{254}, Merck). A mixture of diethyl ether : acetone : chloroform : H_2O (50 : 50 : 40 : 1, by vol.) was used as the developing solvent. R_f values (retardation factors) for FTC-092 and CF_3dUrd were 0.6 and 0.28, respectively. CF_3dUrd was eluted from the plate with 50% methanol, and the radioactivity was measured following the addition of ACS-II (Amersham, Japan) in a Packard 2,000 CA scintillation system.

Preparation of subcellular fractions. Normal male ICR/JCL mice were starved overnight and killed by cervical dislocation. Their livers were perfused in situ with a cold 0.9% NaCl solution and then excised. Subcellular fractions were prepared at 0–4° C by the method of Imai et al. [17]. In a total volume of 1 ml, the reaction mixture contained [2-^{14}C]-FTC-092 (sp. act., 58.7 mCi/mmol), 50 mM TRIS-HCl (pH 7.4), 25 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM NADPH, and a mouse-liver subcellular fraction. The incubation was performed at 37° C for 10 min, and the reaction was terminated by the addition of an equal volume of methanol containing 0.1 *N* HCl. The protein that precipitated was removed by centrifugation at 3,000 *g* for 10 min at 4° C. The amount of CF_3dUrd in the supernatant was measured using the TLC method described above.

Results

Effect of FTC-092 administration at various schedules on tumor growth in mice

The antitumor activity of FTC-092 given to mice on different treatment schedules is shown in Table 1. FTC-092 appeared to be active against S180, a locally growing solid tumor, and its activity showed a significant schedule dependency. The oral administration of FTC-092 produced the highest antitumor activity when carried out consecutively from day 1 to day 9 at a dose lower than that used for a single administration on day 1 or for intermittent treatments on days 1, 5, and 9. The antitumor activity of CF_3dUrd, FT, and FUra also displayed schedule dependency. The consecutive administration of these drugs were most effective (data not shown). On the basis of these observations, we decided to assess the antitumor activity of FTC-092 and other fluorinated pyrimidines using consecutive administration.

Table 1. Influence of the FTC-092 treatment schedule on sarcoma 180 in male ICR mice

Treatment schedule	Dose (mg/kg daily)[a]	Body wt. change (g, days 0–12)	Tumor wt. (g, mean ± SD)	Inhibition (%)
Control	–	+ 10.2	6.16 ± 1.56	
Day 1	900 × 1 (900)	+ 10	4.02 ± 1.55*	35
	450 × 1 (450)	+ 10.7	5.18 ± 1.87	16
Days 1, 5, 9	300 × 3 (900)	+ 7.9	4.27 ± 0.73**	31
	150 × 3 (450)	+ 8.6	5.34 ± 1.79	13
Days 1–9	30 × 9 (270)	+ 5.1	2.48 ± 0.69***	60
	15 × 9 (135)	+ 7	2.81 ± 0.79***	54

Male ICR/JCL mice were implanted subcutaneously on day 0 with 5×10^6 sarcoma 180 cells. The test compounds were given orally on several schedules. For the control, vehicle (0.5% carboxymethyl cellulose solution containing 0.1% Tween-80) was given orally on days 1–9. The percentage of inhibition of tumor growth was evaluated on day 12. Difference from the corresponding control group at $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$

[a] Values in parentheses represent the total dose

Comparison of the antitumor activity of FTC-092, CF_3dUrd, FT, and FUra

The antitumor activity and the therapeutic index of FTC-092 against S180 were compared with those of CF_3dUrd, FT, and FUra (Table 2). Although FTC-092 is a prodrug of CF_3dUrd, it exhibited marked activity at doses lower than those of CF_3dUrd. The ED_{50} value for FTC-092 was 13.3 mg/kg daily, whereas those for CF_3dUrd, FT, and FUra were 64.1, 122, and 28 mg/kg daily, respectively. The therapeutic indices (LD_{10}/ED_{50}) of FTC-092, CF_3dUrd, FT, and FUra were 4.39, 1.7, 1.35, and 1.65, respectively. The therapeutic index of FTC-092 was thus >3-fold that of the parent compound CF_3dUrd. In contrast, the therapeutic indices of FUra and its prodrug FT were quite similar to each other.

The effect of FTC-092 on the survival of mice bearing L1210 was compared with that of CF_3dUrd, FT, and FUra. The ILS_{30} value for FTC-092 was 15.7 mg/kg daily, which was about half that of CF_3dUrd. The therapeutic ratio observed for FTC-092 was 3.82, showing its superiority to FT and FUra. The ILS_{max} (%) data also indicated the superiority of FTC-092.

Table 3. Inhibition of HeLa cell growth by derivatives of CF_3dUrd

Compound	Concentration (μg/ml)				
	10	1	0.1	0.01	IC_{90} (μg/ml)
FTC-092	54 %	5.3%	5.3%	–3.1%	>10
CF_3dUrd	99.8%	98.8%	96.3%	48.7%	0.07
F_3Thy	70.1%	25.8%	16.4%	1.2%	>10
FUra	99.8%	98.9%	81.8%	21 %	0.32

HeLa cells were cultivated in petri dishes containing Eagle's minimum essential medium plus 10% calf serum. Each value represents the average of 3 tests. In the untreated control dishes, 1×10^4 cells that had been seeded on day 0 had proliferated logarithmically to 6.9×10^5 cells by day 7

Effect of FTC-092 on HeLa cells cultured in vitro

The growth-inhibitory activity of FTC-092 and its metabolites against HeLa cells is shown in Table 3. The IC_{90} value (the concentration producing 50% inhibition of cell growth) found was 0.07 μg/ml for CF_3dUrd and >10 μg/ml for FTC-092. Thus, in terms of direct cytotoxicity, the activity of FTC-092 was very low, whereas CF_3dUrd was highly active. F_3Thy, the base of CF_3dUrd, showed only weak activity corresponding to an IC_{90} value of >10 μg/ml.

CF_3dUrd levels in the blood and organs of mice after oral administration of FTC-092 and CF_3dUrd

Mice were given FTC-092 or CF_3dUrd orally at 30 mg/kg, and the concentration of CF_3dUrd in the plasma and organ tissue samples was determined at 0.5, 1, 2, 4, and 8 h after administration (Fig. 2). Following the administration of CF_3dUrd, the maximal plasma concentration of CF_3dUrd (3.13 μg/ml) was reached at 0.5 h and the level rapidly decreased within the first 2 h. Following treatment with FTC-092, the concentration of CF_3dUrd measured in plasma at 0.5 h was lower than that observed after oral dosing with CF_3dUrd, but the level decreased more slowly and remained detectable for 4 h. The concentrations of CF_3dUrd measured in the stomach and small intestine after

Table 2. Comparison of the therapeutic index and the ratios of FTC-092 and fluorinated pyrimidines against sarcoma 180 and L1210 in mice

Drug	Sarcoma 180 (solid type)			L1210 (ascites type)		
	ED_{50}	LD_{10}	Therapeutic index (LD_{10}/ED_{50})	ILS_{30}	ILS_{max}	Therapeutic ratio (ILS_{max}/ILS_{30})
	(mg/kg daily)			(mg/kg daily)		
FTC-092	13.3	58.4	4.39	15.7	60 (86)[a]	3.82
CF_3dUrd	64.1	108.9	1.7	33.2	120 (48)[a]	3.61
FT	122	165.1	1.35	71	120 (52)[a]	1.69
FUra	28	46.1	1.65	17.2	30 (45)[a]	1.72

The antitumor activities of FTC-092 and other fluorinated pyrimidines were assessed following consecutive administration from day 1 to day 9. LD_{10} was calculated from the number of survivors on the 30th day after the first of nine consecutive daily oral doses

[a] ILS_{max} (%) for L1210 cells; the mean survival of the control group was 8.5 ± 0.5 days

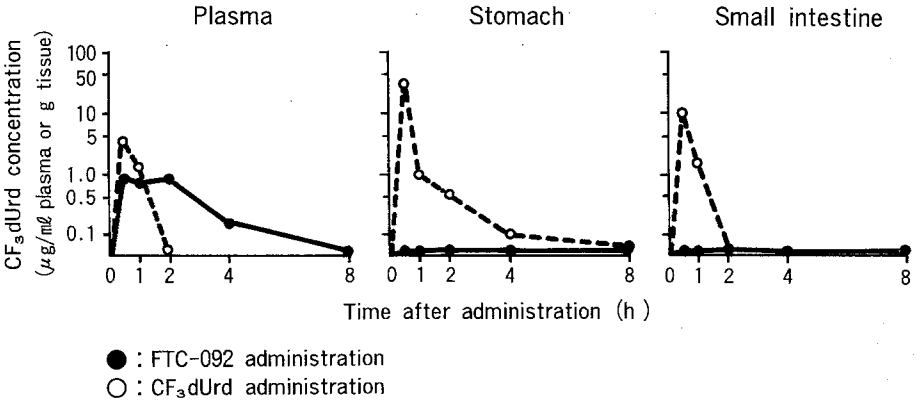

Fig. 2. Comparison of the plasma and tissue levels of CF_3dUrd after oral administration of FTC-092 (30 mg/kg) and CF_3dUrd (30 mg/kg) to ICR/JCL mice

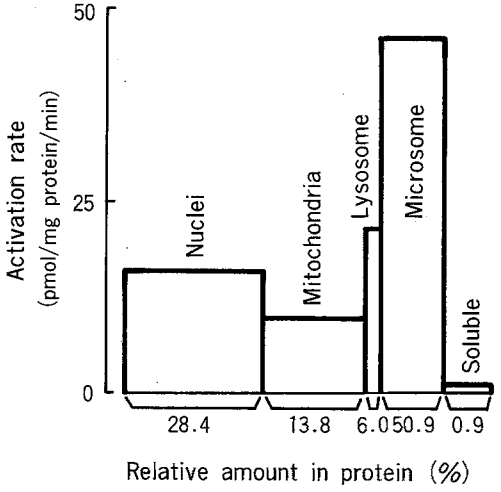

Fig. 3. Activation of FTC-092 to CF_3dUrd in subcellular fractions of mouse liver. FTC-092 (1×10^{-5} M) was incubated with mouse-liver subcellular fractions at 37° C for 10 min in the presence of NADPH. The reaction was linear with time to 10 min

oral administration of CF_3dUrd were 33.71 and 9.57 μg/g tissue, respectively, at 0.5 h, whereas those determined in these organs following the administration of FTC-092 were <0.05 μg/g tissue throughout the entire observation period.

Hydrolysis of FTC-092 in aqueous solution

In 0.1 *N* HCl at 37° C, FTC-092 remained completely stable for 24 h. At a neutral pH of 7, only 23% of the compound was hydrolyzed within 24 h. In the HPLC analysis of the hydrolysis, the product was eluted at a retention time of 15.5 min (see Materials and methods for the experimental conditions), whereas FTC-092 and CF_3dUrd exhibited retention times of 18.9 and 10.9 min, respectively. This hydrolysis product has been tentatively identified as being a 5-COOH derivative of FTC-092 based on mass spectrometry (data not shown). Under conditions that mimic the gastrointestinal tract and tissues, FTC-092 seems to be highly stable.

Organ distribution of the hydrolyzing activity

The enzymic conversion of FTC-092 to CF_3dUrd in mice was examined. The liver homogenate exhibited sufficient activity to convert FTC-092 to CF_3dUrd to an extent of 2.8% following 60 min incubation under the conditions used (see Materials and methods). No significant activity was detected in the other tissues evaluated, including lung, stomach, small intestine, thymus, spleen, kidney, heart, brain, muscle, tumor (S180), and plasma. The conversion of FTC-092 into inactive F_3Thy or 3-*O*-benzyl-deoxyribose was not observed in any of the samples investigated.

Conversion of FTC-092 to CF_3dUrd by the microsomal fraction of mouse liver

Hydrolyzing activity was exhibited mainly by the microsomal fraction (Fig. 3). About 50% of the total activity was found in the microsomal fraction, which also displayed the highest specific activity among the subcellular fractions examined.

Discussion

FTC-092, which had been selected as the best candidate among our new CF_3dUrd analogs, could successfully be given by the oral route. FTC-092 exhibits properties that are quite different from those of the parent compound CF_3dUrd.

First, a much higher degree of antitumor activity is observed for FTC-092, even at lower doses level. Second, the range between the minimal effective dose and the maximal tolerated dose is wide enough to enable the safe use of FTC-092. Third, the amount of CF_3dUrd that is released following the oral administration of FTC-092 is retained in the blood for a long period. This is especially important because the achievement of levels of CF_3dUrd (the parent compound) that are sufficient to produce an antitumor effect requires the use of more complicated treatment schedules (every 3 h daily for 8–13 days or continuous infusion in clinical use) [1]. Fourth, FTC-092 is resistant to the inactivating enzyme thymidine phosphorylase, which degrades CF_3dUrd to F_3Thy, and it remains stable under

both acidic and physiological pH conditions. These properties seem to be important, since gastric juice is acidic and the thymidine phosphorylase activity of the small intestine is higher than that of other organs [16]. Finally, the levels of CF_3dUrd measured in the stomach and small intestine after the oral administration of FTC-092 were significantly lower than those found after CF_3dUrd administration. Since the gastrointestinal toxicity of CF_3dUrd seems to be related to its concentration in the stomach and small intestine, it is important that the concentration of this drug be kept low in these organs.

In the present study, we found that FTC-092 itself is a nonactive agent, but during its gradual biotransformation by the microsomal fraction of the liver, it releases CF_3dUrd as an active compound over a long period. The long-lasting effect of the CF_3dUrd would confer sufficient therapeutic advantages on the former compound. In preliminary studies on the antitumor spectra of FTC-092, the compound was found to be effective against murine solid tumors, including colon adenocarcinoma 38 and melanoma B16, against tumor xenografts of human breast cancer MX-1 and gastric cancer SC-2 in nude mice, and also against various FUra-resistant tumors.

References

1. Ansfield FJ, Ramirez G (1971) Phase I and II studies of 2′-deoxy-5-(trifluoromethyl)-uridine (NSC-75 520). Cancer Chemother Rep 55: 205
2. Ardalan B, Glazer R (1981) An update on the biochemistry of 5-fluorouracil. Cancer Treat Rev 8: 157
3. Blokhina NG, Vozny EK, Garin AM (1972) Results of treatments of malignant tumors with ftorafur. Cancer 30: 390
4. Bosch L, Harbers E, Heidelberger C (1958) Studies on fluorinated pyrimidines: V. Effects on nucleic acid metabolism in vitro. Cancer Res 18: 335
5. Caballero GA, Ausman RK, Quebbeman EJ (1985) Long-term, ambulatory, continuous infusion of 5-FU for the treatment of advanced adenocarcinomas. Cancer Treat Rep 69: 13
6. Carrico CK, Glazer RI (1979) Effects of 5-fluorouracil on the synthesis and translation of polyadenylic acid containing RNA from regenerating rat liver. Cancer Res 39: 3694
7. Danenberg PV, Heidelberger C, Mulkins MA, Peterson AR (1981) Incorporation of 5-fluoro-2′-deoxyuridine into DNA of mammalian tumor cells. Biochem Biophys Res Commun 102: 654
8. Dexter DL, Wolberg WH, Ansfield FJ, Helson L, Heidelberger C (1972) The clinical pharmacology of 5-trifluoromethyl-2′-deoxyuridine. Cancer Res 32: 247
9. Dolnick BJ, Pink JJ (1983) 5-Fluorouracil modulation of dihydrofolate reductase RNA levels in methotrexate resistant KB cells. J Biol Chem 258: 13 299
10. Duschinsky R, Pleven E, Heidelberger C (1957) The synthesis of 5-fluoropyrimidines. J Am Chem Soc 79: 4559
11. Fujiwara Y, Oki T, Heidelberger C (1970) Fluorinated pyrimidines: XXXVII. Effects of 5-trifluoromethyl-2′-deoxyuridine on the synthesis of deoxyribonucleic acid of mammalian cells in culture. Mol Pharmacol 6: 273
12. Hartman KY, Heidelberger C (1961) Studies on fluorinated pyrimidines: XIII. Inhibition of thymidylate synthase. J Biol Chem 236: 3006
13. Heidelberger C, Anderson SW (1964) Fluorinated pyrimidines: XXI. The tumor-inhibitory activity of 5-trifluoromethyl-2′-deoxyuridine. Cancer Res 24: 1979
14. Heidelberger C, Ansfield FJ (1963) Experimental and clinical use of fluorinated pyrimidines in cancer chemotherapy. Cancer Res 23: 1226
15. Heidelberger C, Parsons DG, Remy DC (1964) Synthesis of 5-trifluoromethyluracil and 5-trifluoromethyl-2′-deoxyuridine. J Med Chem 7: 1
16. Ikenaka K, Fukushima M, Nakanura H, Okamoto M, Shirasaka T, Fujii S (1981) Metabolism of pyrimidine nucleotides in various tissues and tumor cells from rodents. Gann 72: 590
17. Imai Y, Ito A, Sato R (1966) Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. J Biochem (Tokyo) 60: 417
18. Langenback RJ, Danenberg PV, Heidelberger C (1972) Thymidylate synthase: mechanism of inhibition by 5-fluoro-2′-deoxy-uridylate. Biochem Biophys Res Commun 48: 1565
19. Major PP, Egan E, Herrick K, Kufe DW (1982) 5-Fluorouracil incorporation in DNA of human breast carcinoma cells. Cancer Res 42: 3005
20. Nakayama C, Wataya Y, Meyer RB, Santi DV (1980) Thymidine phosphorylase. Substrate specificity for 5-substituted 2′-deoxyuridines. J Med Chem 23: 962
21. Nayak R, Nartin D, Stolfi R, Furth J, Spiegelman S (1978) Pyrimidine nucleosides enhance the anticancer activity of FU and augment its incorporation into nuclear RNA. Proc Am Assoc Cancer Res 19: 63
22. Reyes P, Heidelberger C (1965) Fluorinated pyrimidines: XXVI. Mammalian thymidylate synthetase: its mechanism of action and inhibition by fluorinated nucleotides. Mol Pharmacol 1: 14
23. Santi DV, McHenry CS (1972) 5-Fluoro-2′-deoxy-uridylate: covalent complex with thymidylate synthase. Proc Natl Acad Sci USA 69: 1855
24. Seifert P, Baker HL, Reed ML (1975) Comparison of continuously infused 5-fluorouracil with bolus injection in the treatment of patients with colorectal adenocarcinoma. Cancer 36: 123
25. Shah A, MacDonald W, Goldie J, Gudauskas G, Brisebois B (1985) 5-FU infusion in advanced colorectal cancer: a comparison of three dose schedules. Cancer Treat Rep 69: 739
26. Spears CP, Shahinian AH, Moran RG, Heidelberger C, Corbett TH (1982) In vivo kinetics of thymidylate synthase inhibition in 5-fluorouracil-sensitive and -resistant murine colon adenocarcinomas. Cancer Res 43: 450
27. Spears CP, Gustavsson BG, Mitchell MS, et al (1984) Thymidylate synthetase inhibition in malignant tumors and normal liver of patients given intravenous 5-fluorouracil. Cancer Res 44: 4144
28. Swayer RC, Stolfi RL, Martin DS, Spiegelman S (1984) Incorporation of 5-fluorouracil into murine bone marrow DNA in vivo. Cancer Res 44: 1847
29. Umeda M, Heidelberger C (1968) Comparative studies of fluorinated pyrimidines with various cell lines. Cancer Res 28: 2529
30. Wataya Y, Santi DV, Hansch C (1977) Inhibition of *Lactobacillus casei* thymidylate synthetase by 5-substituted 2′-deoxyuridylates. Preliminary quantitative structure-activity relationship. J Med Chem 20: 1469
31. Wilkinson DS, Tlsty TD, Hanas RJ (1975) The inhibition of ribosomal RNA synthesis and maturation in Novikoff hepatoma cells by 5-fluorouridine. Cancer Res 35: 3014
32. Yamashita J, Takeda S, Matsumoto H, Terada T, Unemi N, Yasumoto M (1987) Studies on antitumor agents: VI. Syntheses and antitumor activities of acyl derivatives of 2′-deoxy-5-trifluoromethyluridine. Chem Pharm Bull (Tokyo) 35(5): 2090
33. Yamashita J, Takeda S, Matsumoto H, Unemi N, Yasumoto M (1987) Studies on antitumor agents: VII. Antitumor activities of *O*-alkoxyalkyl derivatives of 2′-deoxy-5-trifluoromethyluridine. Chem Pharm Bull (Tokyo) 35(6): 2373
34. Yamashita J, Takeda S, Matsumoto H, Unemi N, Yasumoto M (1989) Studies on antitumor agents: VIII. Antitumor activities of *O*-alkyl derivatives of 2′-deoxy-5-(trifluoromethyl)uridine and 2′-deoxy-5-fluorouridine. J Med Chem 32: 136