Cancer Chemother. Pharmacol. 4, 83–87 (1980)

Cancer Chemotherapy and Pharmacology

Inhibition of Murine Colon Adenocarcinomas and Lewis Lung Carcinoma by 1-Hexylcarbamoyl-5-fluorouracil

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Summary. *1-Hexylcarbamoyl-5-fluorouracil (HCFU) and its parent compound 5-fluorouracil (5-FU) were tested PO for antitumor activity against mouse colon adenocarcinoma 26 (colon 26), colon adenocarcinoma 38 (colon 38), and Lewis lung carcinoma. The drugs were given orally at 2–4 days intervals for a total of ten doses. 5-FU was moderately active against colons 26 and 38 but not against Lewis lung carcinoma. In this treatment regimen the most impressive antitumor activity was obtained with HCFU against colons 26 and 38, especially colon 38 tumor. At 300 mg HCFU/kg, one out of seven mice inoculated with colon 26 and five out of ten mice inoculated with colon 38 became tumor-free. HCFU, however, was marginally effective in the prolongation of survival time of mice bearing Lewis lung carcinoma.*

5-FU is approximately twice as active as HCFU against cultured cell lines from colon 26, colon 38, and Lewis lung carcinoma. Lewis lung carcinoma cells were most sensitive against HCFU, which is in contrast to the results obtained in the in vivo experiment. The IC_{50} value of HCFU against Lewis lung carcinoma cells was approximately half that against colon 26 and colon 38 cells. This higher sensitivity of Lewis lung cells against HCFU could be explained by the higher cellular uptake of the drug.

Introduction

1-Hexylcarbamoyl-5-fluorouracil (HCFU), a lipophilic masked compound of 5-fluorouracil (5-FU) is a newly synthesized antitumor agent against solid tumors [13]. HCFU was selected by Kuretani and co-workers from a series of 1-alkylcarbamoyl derivatives of 5-FU because of its antitumor activity against L1210 leukemia, C1498 leukemia, ascites sarcoma 180, Ehrlich ascites carcinoma, Nakahara-Fukuoka sarcoma, and adenocarcinoma 755 when administered PO [4–6]. This compound showed superior antitumor activity to 5-FU against Lewis lung carcinoma and B16 melanoma [7]. In addition to its superior antitumor activity, the compound showed lower toxicity in experimental animals when administered PO [8, 9] than did its parent compound, which caused severe gastrointestinal disorders after PO administration [3].

In this study, we found that the antitumor activity of HCFU against colon adenocarcinomas 26 and 38 when administered PO was highly superior to that of 5-FU. The cytocidal effects of HCFU against the cultured cells established from the above three types of murine tumors have been examined.

Materials and Methods

Animals and Tumors

For colon adenocarcinoma 26 (colon 26), adult male BALB/c × DBA/2CR F_1 mice (CDF_1) were used for the experiments, and BALB/c mice were used for carrying tumors. For colon adenocarcinoma 38 (colon 38) and Lewis lung carcinoma, adult male C57BL/6J × DBA/2Cr F_1 mice (BDF_1) were used for the experiments, and C57BL/6J mice were used for carrying tumors. BALB/c, CDF_1, and BDF_1 mice were obtained from Charles River Japan, Inc., Tokyo, Japan and C57BL/6J mice were supplied by Simonsen Labs., Inc., Calif. under the auspices of the NCI, NIH, Bethesda, Md, USA.

Colon 26, colon 38, and Lewis lung carcinoma were supplied by the NCI, NIH, USA and have been passaged in the mice as described above. Tumors were implanted SC for the experiments.

Evaluation of Antitumor Activity

HCFU (Mitsui Pharmaceutical Inc., Tokyo, Japan) and 5-FU (Sigma Chemical Company, St. Louis, Mo.) were administered PO as HCFU administered PO showed a similar antitumor activity and a higher therapeutic ratio (ILS_{max}/ILS_{30}) in the L1210 system to 5-FU administered PO or IP [4]. Duration of the treatments for the tumors was chosen to give drugs a total of ten times from the day after tumor inoculation, based on the lifespan of control mice, so that drugs were

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0344-5704/80/0004/0083/$ 01.00

given PO at 2-day, 3-day, and 4-day intervals against colon 26, Lewis lung carcinoma, and colon 38, respectively. Antitumor activity was evaluated by: (a) Percentage increase in host lifespan (%ILS = T/C% – 100). Tumor-free survivors were excluded from these calculations; (b) Tumor growth inhibition. The average tumor weights of the treated and control groups were monitored regularly.

Drug Treatment of the Cultured Cells

Tumor cells for culture were prepared from tumor tissues by the digestion with trypsin-EDTA (Grand Island Biological). Cells were subcultured ten times and used for experiments. Cells were grown in plastic dishes (Lux Scientific) at 37° C in a humidified atmosphere with 5% CO_2. The initial cell density of each cell line was 1.5×10^5 cells per dish (diameter, 60 mm), which contained 3 ml RPMI 1640 medium with 10% fetal bovine serum. Twenty-four hours after the cells were split, graded concentrations of HCFU and 5-FU were added. Two dishes were used for each drug concentration. At 1 h after the addition of drugs, the medium containing drug was removed and the cell layer was washed twice with 3 ml medium each time, and the cells were further cultivated with 3 ml medium. At 48 h after drug treatment the cells were enumerated with a Coulter counter as described previously [14]. The IC_{50} (concentration of drug required for 50% inhibition of the cell growth) of each drug was obtained by plotting the logarithm of the drug concentration versus the growth rate (percentage of control) of the treated cells.

Uptake of HCFU in Cultured Cells

Cells were seeded at 5×10^5 in 100-mm plastic dishes (Lux Scientific) each containing 10 ml of RPMI 1640 medium with 10% fetal bovine serum. At 24 h after seeding the cells, the radioactive ^{14}C-labeled HCFU (New England Nuclear: 15.5 or 39.0 μM; specific activity: 0.9 mCi/mmol) was added to the dishes. At 1 h after the addition of HCFU, the medium was removed and the cells were washed three times with RPMI 1640 medium (10 ml). The intracellular uptake of HCFU was measured as described previously [14].

Results

Effects of HCFU and 5-FU on the Survival Time of Tumor-bearing Mice

Antitumor effects of HCFU and 5-FU against SC implanted colon 26, colon 38, and Lewis lung carcinoma are summarized in Table 1. HCFU was most effective against SC transplanted colon 26 at 300 mg/kg when administered PO, and one of the seven treated mice was tumor-free on day 70 after tumor transplantation. HCFU at 200 mg/kg was also effective; however, at 400 or 500 mg/kg the drug seemed to be rather toxic and did not show significant antitumor activity. 5-FU showed the highest chemotherapeutic activity at 30 mg/kg when administered PO.

HCFU also showed highly superior chemotherapeutic activity against colon 38. At 300 mg/kg, five of ten treated mice were tumor-free for 100 days after tumor transplantation and the ILS of deceased mice was 123%. At 200 mg/kg and at 100 mg/kg, two and one, respectively, of the ten treated mice were tumor-free for 100 days after inoculation. Of the tumors examined, colon 38 was the most sensitive against HCFU. 5-FU showed maximum activity at 30 or 50 mg/kg.

HCFU, however, did not show a significant antitumor effect on the survival time of the mice bearing Lewis lung carcinoma. The ILS at 300 mg/kg was 38% and no effect was observed at 200 and 100 mg/kg.

Inhibition of Tumor Growth In Vivo by HCFU and 5-FU

The mean volumes of various tumors treated PO with HCFU and 5-FU are shown in Fig. 1. HCFU was moderately active against colon 26. At 300 mg HCFU/kg growth of the tumor was well inhibited. As reported for various colon tumors by Schabel's group [2], we measured the time for the average tumor volume to reach 750 mg for the evaluation of antitumor activity of the drug. Time taken to reach 750 mg for the average colon 26 tumor volume was 18 days in the control group, while in the treated group with 300, 200, and 100 mg HCFU/kg it was 43.0, 21.5, and 17.0 days, respectively. The differences between these values are described in Table 1 as T–C values.

The most sensitive tumor to HCFU was colon 38. Time taken to reach an average tumor volume of 750 mg in the control group was 12.5 days, while in the treated group with 300, 200, and 100 mg HCFU/kg the time was 57.0, 31.5, and 17.5 days, respectively. T–C values are described in Table 1.

Although HCFU did not produce a significant effect on the survival time of mice bearing Lewis lung carcinoma, tumor growth was significantly inhibited by HCFU at 300 mg/kg. At this dosage, growth was well inhibited until day 20, when two-thirds of the treatment schedule had been completed. However, the tumor regained its growth thereafter, growing at almost the same rate as the control tumors. Time taken to reach 750 mg for the control group was 11 days, while the time taken in the group treated with HCFU at 300 mg and 200 mg was 24 and 15 days. respectively. T–C values are shown in Table 1.

Cytotoxic Effects of HCFU and 5-FU Against Cultured Tumor Cells

Growth-inhibitory effects of HCFU and 5-FU against cultured colon 26, colon 38, and Lewis lung carcinoma cells are shown in Fig. 2. 5-FU was more toxic against cultured cells than was HCFU, as the latter is a depot form of

Table 1. Antitumor effects of HCFU and 5-FU against SC implanted mouse tumors

Tumor	Drug	Dose (mg/kg)	MST[a] (day)	ILS[a] (%)	T–C value[b] (days)	Tumor-free survivors[c]
Colon 26	HCFU	500	18.0	0	–[d]	0/7
		400	20.5	14	–[d]	0/7
		300	53.8	199	25	1/7
		200	33.5	86	3.5	0/7
		100	19.0	6	− 1.0	0/7
	5-FU	75	16.0	− 7	0	0/7
		50	28.5	58	12	0/7
		30	33.5	86	4	0/7
		15	18.2	1	0	0/7
	Control	–	18.0	–	–	0/7
Colon 38	HCFU	300	73.5	123	44.5	5/10
		200	59.0	79	19.0	2/10
		100	47.5	40	5.0	1/10
	5-FU	50	47.5	40	13.0	0/10
		30	47.5	40	8.5	0/10
		15	40.0	18	− 3.5	0/10
	Control	–	34.0	–	–	0/10
Lewis lung carcinoma	HCFU	300	37.0	38	13.0	0/10
		200	27.8	4	4.0	0/10
		100	27.7	3	− 0.5	0/10
	5-FU	50	28.1	5	2.5	0/10
		30	24.0	− 10	0.5	0/10
		15	28.3	6	0.5	0/10
	Control	–	26.8	–	–	0/10

[a] Abbreviations used were: MST, median survival time; ILS, increase in lifespan
[b] T–C value (days) = time required for the treatment group tumor (the average value) to reach 750 mg, minus the time required for the control group tumors to grow to the same size, as reported in [2]
[c] Duration of experiments was 70, 100, and 100 days for colon 26, colon 38, and Lewis lung carcinoma, respectively. Tumor-free survivors were counted on the last day of each experiment
[d] Tumor was not palpable during observation period

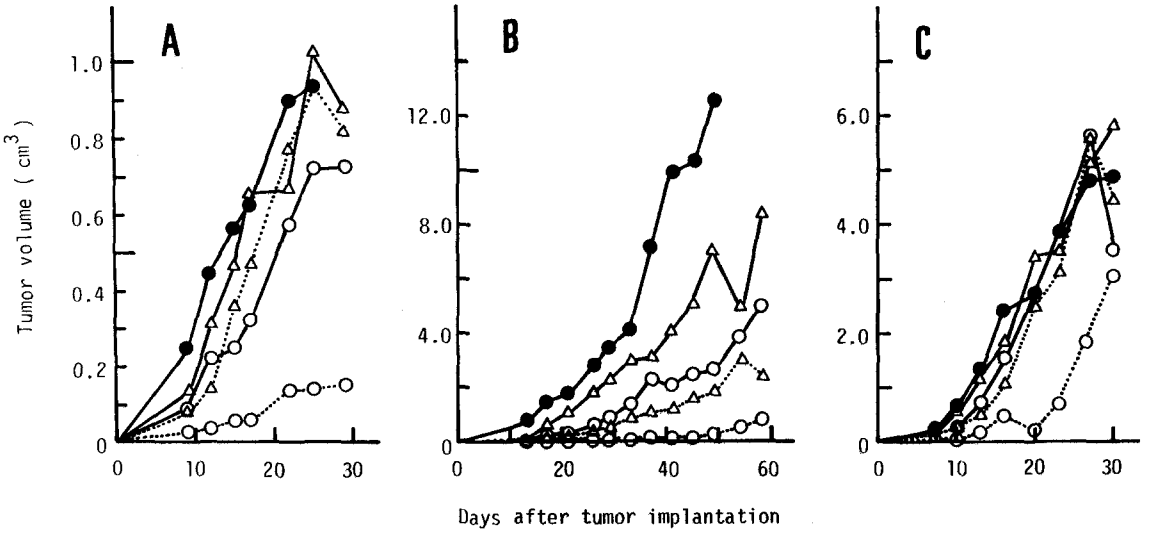

Fig. 1 A–C. Tumor volumes of (**A**) colon 26, (**B**) colon 38, and (**C**) Lewis lung carcinoma treated with HCFU and 5-FU. Tumor-inoculated mice were given PO HCFU at 300 (○- - - - -○) and 200 (△- - - - -△) mg/kg and 5-FU at 50 (○——○) and 30 (△——△) mg/kg. The closed circle (●——●) represents the tumor volume of the control group. HCFU and 5-FU were given in ten doses from day 1, at 2-day intervals for colon 26, 4-day intervals for colon 38, and 3-day intervals for Lewis lung carcinoma

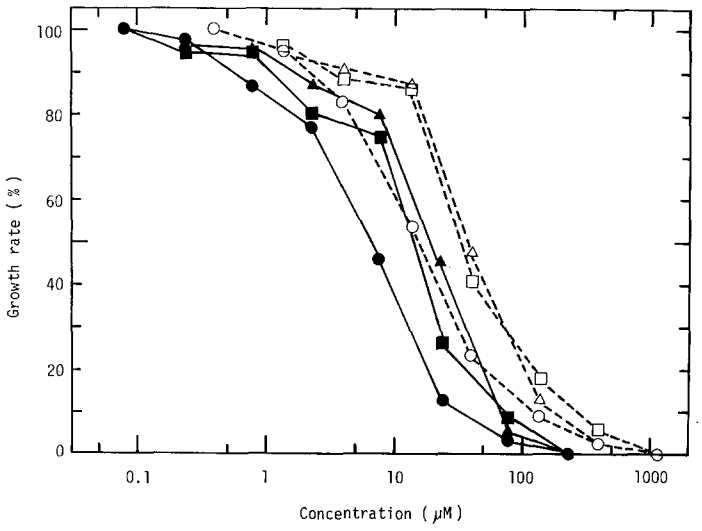

Fig. 2. Growth-inhibitory effects of HCFU and 5-FU on cultured colon 26, colon 38, and Lewis lung carcinoma cells. Cultured cells were treated for 1 h with graded concentrations of HCFU and 5-FU 24 h after seeding the cells at 1.5×10^5 cells in 60-mm dishes. Cell numbers were counted 2 days after the drug treatment. Growth of colon 26 (△- - - -△), colon 38 (□- - - - -□), and Lewis lung carcinoma cells (○- - - - -○) in the presence of HCFU, and colon 26 (▲——▲), colon 38 (■——■), and Lewis lung cells (●——●) in the presence of 5-FU

Table 2. Uptake of HCFU by cultured colon 26, colon 38, and Lewis lung carcinoma cells

Cells	HCFU concentration (μ*M*)	Cellular uptake of HCFU (pmol/10^6 cells)
Colon 26	15.5	131
	39.0	241
Colon 38	15.5	193
	39.0	325
Lewis lung carcinoma	15.5	240
	39.0	431

5-FU and an activation process is needed before its action. The IC_{50} values of HCFU against colon 26, colon 38, and Lewis lung carcinoma were 39.0, 32.0, and 15.5 μ*M* respectively, while the values of 5-FU against these tumors were 20.0, 15.0, and 6.5 μ*M* respectively. The values of IC_{50} of HCFU/IC_{50} of 5-FU were 2.0, 2.1, and 2.4 for colon 26, colon 38, and Lewis lung carcinoma cells, respectively. The similarity of these values may suggest a similar activation velocity of HCFU to 5-FU in these cultured cell lines.

Uptake of HCFU by Cultured Tumor Cells

The cellular uptake of HCFU is shown in Table 2. Drug concentrations were 15.5 and 39.0 μ*M*, and these were the IC_{50} doses against Lewis lung carcinoma cells and colon 26 cells, respectively. The highest uptake of HCFU was observed for Lewis lung carcinoma cells, and 240 pmol and 431 pmol HCFU were found in 10^6 Lewis lung cells at drug concentrations of 15.5 and 39.0 μ*M*, respectively. The uptake of HCFU into colon 26 cells was 131 and 241 pmol per 10^6 cells at each drug concentration, and these values were approximately half those obtained for Lewis lung carcinoma. In addition, similar extents of drug uptake occurred at each IC_{50} for each cell line. These results probably indicate that the cytotoxicity of HCFU against Lewis lung and colon 26 cells might be explained by the extent of uptake of the drug.

Discussion

In this study, we found there was a higher sensitivity of HCFU against colon adenocarcinomas, especially colon 38. In in vitro experiments 5-FU was more cytotoxic than HCFU. HCFU is a depot form of 5-FU and an activation process is needed for its action in cultured cells. In in vivo experiments, however, HCFU might show a sustained release of the drug to provide a sufficient therapeutic level of 5-FU over a prolonged period of time, revealing a marked therapeutic activity against colon tumors.

In an in vitro experiment, Lewis lung carcinoma cells were the most sensitive of the cell lines examined to HCFU; however, in an in vivo experiment this tumor was not so sensitive to HCFU. There are three possible explanations for this discrepancy. First of all, the distribution of active drug in animal is different among the three tumor systems, and the concentration of drug was lower in Lewis lung carcinoma than in colon 38. In in vivo experiments with three tumor lines, however, the site of tumor inoculation was SC and HCFU was given PO. It is thus rather difficult to speculate that the drug distribution is different among the three tumor systems. The second possible explanation is that the extent of tumor metastasis is different among these lines. Lewis lung carcinoma metastasizes well to the lung [10, 12] and colon 26 is a highly metastatic tumor among mouse colon tumors [1, 2]. In contrast, the incidence of tumor metastasis for colon 38 is

reported to be very low [1, 2]. The third explanation is the possible appearance or amplification of resistant cells against HCFU. The accelerated growth of Lewis lung carcinoma when two-thirds of the treatment schedule has been completed (Fig. 1) might be interpreted with reference to this explanation. Changes in cell population leading to drug resistance in tumors are widely observed [11]. Further study is needed to explain the above discrepancy.

Acknowledgements: We thank Mitsui Pharmaceuticals, Inc., for supplying HCFU. This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

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Received November 15, 1979/Accepted January 2, 1980