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Potentiation of antitumor activity of 1-phthalidyl 5-fluorouracil by acetazolamide

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Summary. The effect of acetazolamide (A.A.) on the antineoplastic activity of 1-phthalidyl 5-fluorouracil (PH-FU) against rat and mouse solid tumors was examined. A.A., an inhibitor of liver PH-FU hydrolase, had no antitumor activity but greatly enhanced the activity of PH-FU when coadministered. The potentiation was evaluated in terms of suppression of tumor growth and prolongation of the life-span of tumor-bearing animals. Studies also revealed that A.A. elevated the concentration of PH-FU in tumor tissues, where 5-fluorouracil is slowly liberated from PH-FU. The results are consistent with the hypothesis that A.A. prevents enzymic degradation of PH-FU in the liver and promotes its distribution into target organs.

Introduction

5-Fluorouracil (5-FU) interferes with the metabolism of pyrimidines and acts cytotoxically on tumor cells as well as host cells [3, 5, 12]. To reduce the side effects of 5-FU and facilitate its availability in the region where tumor cells are proliferating, various prodrugs of 5-FU have been developed [1, 2, 7, 11]. One of them, PH-FU (1-phthalidyl 5-fluorouracil), is absorbed efficiently from the gastrointestinal tract and generates 5-FU in tissues by the action of hydrolase(s), with the highest activity in the liver [6]. Since a large portion of the 5-FU released from PH-FU in the liver is expected to be metabolized to smaller molecules for disposal [8, 9] inhibitors of the hydrolase should elevate the PH-FU concentration in the body fluid and promote its distribution in the tissues. Coadministration of a hydrolase inhibitor, acetazolamide (A.A.), has been shown to elevate the PH-FU level in rat plasma [6]. The present study revealed that A.A. remarkably enhanced the antitumor activity of PH-FU in tumor-bearing rats and mice, but not the activity of 5-FU. In agreement with these findings, the concentration of PH-FU in tumor cells was higher when PH-FU and A.A. were ingested in combination.

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Abbreviations: A.A., acetazolamide; CMC, carboxymethyl cellulose; 5-FU, 5-fluorouracil; HPLC, high-performance liquid chromatography; PH-FU, 1-phthalidyl 5-fluorouracil

Materials and methods

Chemicals. PH-FU was provided by Dr S. Kamata of Shionogi Research Laboratory. 5-FU and A.A. were purchased from Sigma.

Animals. Female Wistar rats 4–5 weeks old, and 5-week-old female D.S. mice were maintained at 23±2 °C with a cycle of 12 h light and 12 h dark and allowed free access to laboratory chow and water. To test antitumor activity, 10^6 cells of Yoshida sarcoma and 5×10^6 cells of Crocker sarcoma 180 were implanted in the backs of rats and mice, respectively.

Treatment. Drug treatment of the animals was started 24 h after the inoculation of tumor cells. PH-FU suspended in 0.5% CMC (70 mg/ml) was administered PO to rats and mice for 4 consecutive days at daily dosages of 350 mg/kg and 150 mg/kg, respectively. 5-FU was administered either PO or IP at dosages of 40 mg/kg and 20 mg/kg, respectively. A.A. was suspended in 0.5% CMC and administered PO (5 ml/kg) 15 min before PH-FU.

Antitumor activity. Rats and mice were killed by cervical dislocation 7 days and 10 days after implantation of tumor cells, respectively, and the tumors in the backs were excised and weighed. Yoshida sarcoma inoculated into the backs of rats became palpable in 3 days and then grew rapidly to about 5 g if the animals were not treated with drugs. Crocker sarcoma 180 grew to nearly 1.2 g by 10 days after transplantation to mice.

Determination of PH-FU in tumor tissues. Five days after SC inoculation of Yoshida sarcoma, PH-FU (350 mg/kg) or PH-FU and A.A. (50 mg/kg) were administered PO to rats, which were sacrificed at 1-h intervals. The tumors in the backs were excised and homogenized with Ultra-Turrux (Janke & Kunkel KG) at 4 °C in 5 vol. (W/V) 0.9% NaCl. PH-FU in 4 ml homogenate was extracted by vigorous shaking with 32 ml ethyl acetate, after which 24-ml portions were evaporated to dryness in vacuo. The residue was dissolved in 0.3 ml 50 m*M* KH_2PO_4-CH_3CN (3 : 1) which was subjected to HPLC as described before using a Nucleosil 10 C_{18} column (0.4 × 30 cm) and 50 m*M* KH_2PO_4-CH_3CN (3 : 1) as the mobile phase [6].

Results

Antitumor activity of PH-FU and A.A.

As shown in Table 1, the growth of rat tumors was significantly suppressed by PH-FU. (The experiments were repeated eight times and typical results are shown; enhancement by A.A. was seen to be reproducible.) Although A.A. (200 mg/kg) did not show any effects on tumor growth and body weight gain at all, it enhanced the antitumor activity of PH-FU in a dose-dependent manner. A similar effect of A.A. was observed when PH-FU was administered to tumor-bearing mice (Table 2), suggesting that the cooperative action of A.A. can be seen generally in murine tumors. This agrees with previous findings that A.A. inhibits liver PH-FU hydrolase in both rats and mice [6]. The difference in the effective dosage of A.A. between rat and mouse may be due to the different sensitivities of the liver hydrolases to A.A. in the two rodents. Sulfonamides, like sulfanilamide or sulfathiazole, enhance the activity of PH-FU, though less potently than A.A. (data not shown).

Antitumor activity of 5-FU

While IP injection of 5-FU markedly suppressed tumor growth, A.A. administered IP or PO did not stimulate this activity (Table 3). In a separate experiment, 5-FU was given PO (40 mg/kg/day) for 4 days; tumor growth was

Table 1. Antitumor activity of PH-FU and coadministration of PH-FU and A.A. on Yoshida sarcoma implanted in the backs of Wistar rats

Treatment (4 days)	Tumor weight (g)
–	5.3 ± 0.33
A.A. (200 mg/kg)	5.2 ± 0.27
PH-FU	3.0 ± 0.16
PH-FU + A.A. (3 mg/kg)	1.5 ± 0.37**
PH-FU + A.A. (12 mg/kg)	1.2 ± 0.30**
PH-FU + A.A. (25 mg/kg)	0.4 ± 0.09**

Different amounts of A.A. were administered PO 15 min before PH-FU (350 mg/kg, PO). Average body weight at the start of the experiment was 109 ± 1.3 g. Values given are means ± SE ($n=5$)
** Statistically significant at $P < 0.01$ against PH-FU

Table 2. Treatment of Crocker sarcoma 180 inoculated in the back of D.S. mice with PH-FU or with PH-FU and A.A.

Treatment (4 days)	Tumor weight (g)
–	1.21 ± 0.14
PH-FU	0.60 ± 0.07
PH-FU + A.A. (50 mg/kg)	0.37 ± 0.03*
PH-FU + A.A. (100 mg/kg)	0.29 ± 0.05**

PH-FU suspension (70 mg/ml) was administered PO at 150 mg/kg and A.A. was administered PO 15 min before PH-FU. Average body weight of the mice used was 21.7 ± 0.2 g. Values given are means ± SE ($n = 5$)
* $P < 0.05$;
** $P < 0.01$ (statistically significant against PH-FU)

Table 3. Antitumor activity of 5-FU and effect of A.A. coadministration on Yoshida sarcoma implanted in the backs of Wistar rats

Treatment (4 days)	Tumor weight (g)
–	4.3 ± 0.07
5-FU	1.6 ± 0.07
5-FU + A.A. (PO)	2.0 ± 0.27
5-FU + A.A. (IP)	1.7 ± 0.14

Female Wistar rats were 4 weeks old and weighed 89.3 ± 0.9 g. 5-FU was injected IP (20 mg/kg) and A.A. (50 mg/kg) was administered either PO or IP 15 min before 5-FU injection. Values given are means ± SE ($n = 5$)
No significant differences were found with Student's t-test

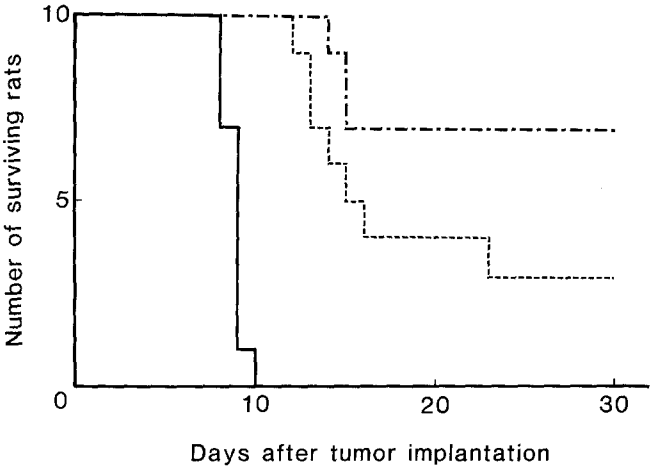

Fig. 1. Survival of rats with 10^6 Yoshida sarcoma implanted into their backs. The rats were treated with drugs for 4 consecutive days 24 h after tumor inoculation. Group 1 (———) received 0.5% CMC PO; group 2 (------) received PH-FU PO (350 mg/kg); and group 3 (·····) received PH-FU and A.A. (25 mg/kg) PO

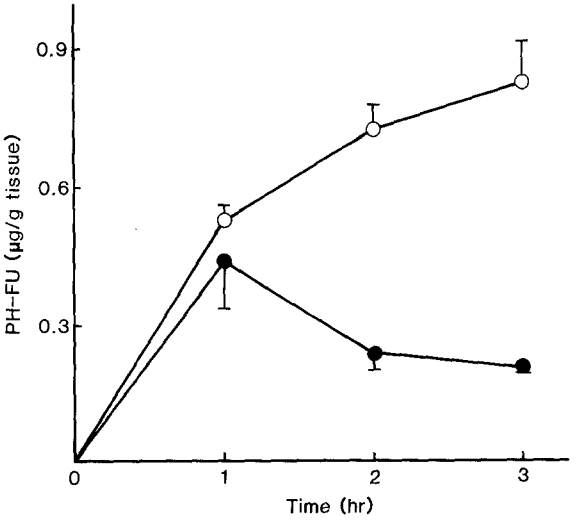

Fig. 2. Concentration of PH-FU in rat solid tumors after administration of PH-FU (●———●) or PH-FU and A.A. (○———○) PO. Drugs were ingested 5 days after implantation of 10^6 Yoshida sarcoma cells, and the amount of PH-FU was determined at 1-h intervals by HPLC. Values are means ± SD ($n=3$)

suppressed by 55%, but here also the coadministration of A.A. did not enhance the antitumor activity of 5-FU (data not shown).

Life-span of tumor-bearing rats

Rats with Yoshida sarcoma implanted in their backs were maintained for a maximum of 60 days. Without drug treatment all rats died within 10 days, while 30% of those treated with PH-FU survived during the period of observation (Fig. 1). When treated with PH-FU and A.A. as many as 70% of the rats survived, with normal body weight gain. Complete regression of tumors was confirmed at 60 days.

Distribution of PH-FU in tumors

The amount of PH-FU in rat tumors was determined by HPLC. As shown in Fig. 2, a higher amount of PH-FU was found in the tumor tissue on a grams per tissue weight basis when PH-FU and A.A. were coadministered.

Discussion

PH-FU is characterized by its antineoplastic activity in solid tumors when administered PO, together with low toxicity to the gastrointestinal tract [4, 10]. The drug liberates 5-FU in various tissues (e.g., liver, kidney) rapidly via enzymic hydrolysis, and slowly in body fluid through a nonenzymic process [6]. Although PH-FU is absorbed efficiently from the intestine, its blood concentration is relatively low and the duration is short because of its elimination by the liver [8, 9]. To maintain a higher level of the drug in body fluids, a modulation of PH-FU degradation in the liver was attempted in this study. As anticipated, A.A. suppressed the first-pass effect of PH-FU in the liver, and as a result promoted drug distribution into the target organs. Since PH-FU decomposed into 5-FU in the buffer of physiological pH at 37 °C (approx. 0.7 nmol/min), the drug accumulated in tumor tissue liberated an effective amount of 5-FU to prevent cell growth. Another possibility is that PH-FU acts directly to prevent the development of tumor cells. Further studies along these lines are in progress in our laboratory. Although attention should be paid to the side effects of the two agents when coadministered, our study does indicate that A.A. potentiates the cytotoxic activity of PH-FU in certain tumors.

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