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PHOSPHORIBOSYL PYROPHOSPHATE, POOL SIZE AND TISSUE LEVELS AS A DETERMINANT OF 5-FLUOROURACIL RESPONSE IN MURINE COLONIC ADENOCARCINOMAS

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Abstract—The antitumor activity of 5-fluorouracil (FUra) in four murine colonic adenocarcinomas was correlated with the basal pool sizes of phosphoribosyl pyrophosphate (PRPP). Mice inoculated with 20–30 mg fragments of murine colonic adenocarcinomas 26 and 38 (FUra-sensitive), and 51 and 11A (FUra-resistant), were treated approximately 2 weeks later with FUra (200 mg/kg, i.p.). In mice bearing adenocarcinomas 26 and 38, intravenous administrations on days 3, 10 and 17 of the highest nontoxic dose of 73 mg/kg produced a tumor growth delay of 14.8 and 11.8 days respectively. In contrast, mice bearing colonic adenocarcinomas 51 and 11A, treated with the same dose schedule of FUra, demonstrated a tumor growth delay of 5 and 5.4 days respectively. The basal levels of PRPP in FUra-sensitive tumors 26 and 38 were 8.7 and 4.0 μM, whereas those in FUra-resistant tumors 11A and 51 were 2.4 and 2.8 μM respectively. Two hours after i.p. injection of FUra (200 mg/kg), FUra-sensitive tumors (26 and 38) showed a substantial reduction in basal levels of PRPP to 1.30 and 2.80 μM respectively; FUra-resistant tumors (51 and 11A) demonstrated a significantly smaller decrease in basal pool size levels. At 24 hr there was a full restitution of intratumoral PRPP to the previous basal levels. Four hours following i.p. injection of FUra at 200 mg/kg, the group of enzymes which could perturb PRPP pool size were assayed, namely PRPP synthetase (EC 2.7.6.1), hypoxanthine-guanine phosphoribosyl transferase (EC 2.4.2.8), adenine phosphoribosyl transferase (EC 2.4.2.7), and orotate phosphoribosyl transferase (EC 2.4.2.10). The specific activity of PRPP synthetase in the FUra-sensitive line (colon tumor 26) was 3-fold higher than in the FUra-resistant tumors examined (colon 51 and 11A); moreover, only in the sensitive line was the specific activity of orotate phosphoribosyl transferase increased significantly following treatment with FUra. Thus, these data suggest that measurement of the basal intratumoral PRPP levels, together with the determination of specific activities of PRPP synthetase and oratate phosphoribosyl transferase, should be tested for its potential clinical application as a means of selecting patients with gastrointestinal and breast carcinomas to be treated with FUra.

Since its synthesis in 1957, the antimetabolite, FUra¶, has played a major role in the treatment of gastrointestinal cancer [1]. However, it is recognized that only 20% of patients with chronic cancer treated with this agent will achieve an objective response [2]. Selection of patients for therapy with FUra remains an exercise in clinical empiricism: there is no simple test that has been accepted as a predictive index for response. The result from our own, and other, laboratories demonstrate that the peak value of the intratumoral concentration of FdUMP can play a role in predicting tumor responsiveness to FUra [3–9]. It has been demonstrated that FUTP and FdUMP are the active metabolites of FUra [10–15]. FdUMP forms a covalent complex with the thymidylate synthetase in the presence of 5,10-CH_2H_4 folate [16, 17]. Thymidylate synthetase, the target enzyme, catalyzes the methylation of the natural substrate, dUMP, to dTMP for DNA synthesis [18, 19]. Moreover, FUMP, when phosphorylated to FUTP, can potentially be incorporated into RNA and thus inhibit RNA and DNA synthesis [10, 20]. The present study was conducted to correlate the tissue level of an important sugar phosphate, i.e. phosphoribosyl pyrophosphate (PRPP), with the antitumor activity of FUra in four chemically induced murine colonic adenocarcinomas. The results demonstrate that the basal PRPP pool levels and specific activities of PRPP synthetase and orotate phosphoribosyl transferase can serve as a useful predictive system for determining *in vitro* tumor sensitivity or FUra, at least in murine adenocarcinomas with known sensitivity and resistance to this agent. A similar study has been done on the effects of glu-

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¶ Abbreviations: FUra, 5-fluorouracil (NSC-19893); PRPP, phosphoribosyl pyrophosphate; FdUMP, 5-fluoro-2′-deoxyuridine 5′-monophosphate; dUMP, deoxyuridine 5′-monophosphate; dTMP, deoxythymidine 5′-monophosphate; OPRTase, orotate phosphoribosyl transferase; R-5-P, ribose-5-phosphate, DTT, dithiothreitol; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; and APRT, adenine phosphoribosyl transferase.

Table 1. Effect of 5-fluorouracil on murine colonic adenocarcinomas*

Tumor*	T−C value†	Log_{10} kill/dose‡	Activity rating to FUra§
26	14.8	0.8	+++
38	11.8	0.4	+
51	5.0	0.2	−
11A	5.4	0.2	−

* Tumor fragments (30 mg) were implanted subcutaneously on day 0 of the experiment. FUra was administered i.v. 73 mg/kg on days 3, 10 and 17.

† Tumor growth delay, treated minus control (T − C in days) = the time required for the treatment group tumors (median of 10) to reach a predetermined size (750–1250 mg) minus the time required for the control group tumors (median of 10) to grow to the same size. Tumor-free survivors were excluded from these calculations.

‡ Log_{10} cell kill/dose $= \dfrac{T - C \text{ value}}{(3.32) \times (Td) \times (\text{no. of doses})}$.

Td = the exponential tumor value doubling time.

§Activity rating	Log_{10} net	Kill gross
++++	>2.0	>2.8
+++	0.8–2.0	2.0–2.8
++		1.3–1.9
+		0.7–1.2
−		<0.7

tamine analogues on enzymes that regulate PRPP levels in P388 leukemia cells and murine colonic adenocarcinomas, *in vivo* [21].

MATERIALS AND METHODS

[2-^{14}C]Fluorouracil (9.1 mCi/mmole) was obtained from SRI International, Stanford, CA. [U-^{14}C]Adenine (276 mCi/mmole) [^{14}C-carboxyl]orotic acid (41.3 mCi/mmole), and [8-^{14}C]hypoxanthine (55.8 mCi/mmole) were obtained from the Amersham Corp., Arlington heights, IL. Aquasol scintillation fluid was purchased from the New England Nuclear Corp., Boston, MA. PRPP, ATP, IMP, AMP, and $MgCl_2$ were obtained from the Sigma Chemical Co., St. Louis, MO. Mouse colon adenocarcinomas 26 and 38 (FUra-sensitive) and 51 and 11A (FUra-resistant) were induced with *N*-methyl-*N*-nitrosourethane [22]. Protein determinations were made with Bio-Rad dye binding reagent (Bio-Rad Laboratories, Richmond, CA) [23]. All four murine tumors were carried as solid tumors. Colonic tumors 11A, 51 and 26 were maintained in BALB-C mice, the host of origin, while the colonic tumor 38 was carried in C57BL/6 mice. The procedure for inoculation of the tumor was as follows. The tumor, 0.5 cm in diameter, was carefully dissected from mice bearing this specific line. The isolated tumors were minced with scissors into small fragments in a sterile, physiological saline. The tumor fragments, weighing 20–30 mg, were implanted subcutaneously with a trocar into new hosts.

Preparation of tumor homogenates for PRPP determination. Mice bearing subcutaneous colonic tumors were administered 200 mg/kg of FUra or saline i.p. At 2, 4, 8, 12 or 24 hr after drug administration, five animals were killed at each time point. Animals were anesthetized with ether, and tumors were rapidly removed and homogenized in 5% perchloric acid (PCA) (tumor:PAC, 1:3, w/v). To each ml of homogenate were added 50 μl of 1 M potassium phosphate (pH 8.5) and 70 μl of 40% KOH to bring it to pH 7–7.2. The neutralized homogenates were spun at 12,000 g for 10 sec in a Eppendorf microcentrifuge, and immediately frozen in dry ice until the supernatant fractions were to be assayed for PRPP. The PRPP and PRPP synthetase assays, the hypoxanthine, adenine, and orotate phosphoribosyl transferase assays, and the preparation of tumor homogenates for the enzyme assays were all performed as in the previous paper [21].

RESULTS

The effects of FUra administration on the tumor growth of four colonic adenocarcinomas are shown

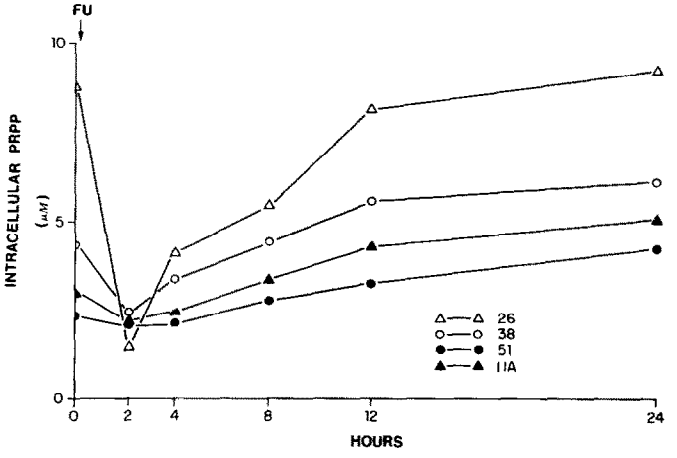

Fig. 1. PRPP time course and dose response. Mice bearing subcutaneous colonic tumors were administered 200 mg/kg of FUra or saline i.p., at 2, 4, 8, 12 and 24 hr. Five animals were killed at each time point, and the tumors were rapidly removed and homogenized in 5% PCA and subsequently adjusted with 40% KOH to pH 7.0. The supernatant fractions were used to determine PRPP content.

in Table 1. Intravenous administration of FUra at 73 mg·kg^{-1}·dose^{-1} on days 3, 10 and 17 produced 2- to 3-fold increases in tumor growth delay in FUra-sensitive compared with resistant lines. In concert with this finding, the $\log_{10}$ kill per dose was 2- to 4-fold higher in the sensitive versus resistant lines. Based on the FUra $\log_{10}$ kill, an activity rating was mathematically calculated, and a rate was given to each colonic carcinoma regarding its sensitivity or resistance to FUra.

Intratumoral PRPP concentration. Basal levels or PRPP in FUra-sensitive tumors, 26 and 38, were 8.7 ± 2.1 and 4.0 ± 1.6 μM, respectively (similar to values in [21]), as compared with the FUra-resistant tumors, 11A and 51, which were 2.4 ± 1.1 and 2.8 ± 1.5 μM, respectively (Fig. 1). Intraperitoneal administration of FUra at 200 mg/kg produced a rapid decrease in the PRPP pool size to 1.30 ± 0.7 μM in the tumor most sensitive to FUra (85%), colon line 26. In concert with this finding, a tumor less sensitive to FUra, i.e. colonic tumor 38, demonstrated a 37.5% reduction in the basal levels or PRPP. FUra-resistant tumors, 51 and 11A, demonstrated 8 and 17% decrease, respectively, in basal levels of the concentration of PRPP (Fig. 1). Interestingly, FUra treatment caused a rebound in the PRPP pool sizes in the resistant tumors and at 24 hr PRPP levels were higher than basal levels in the resistant tumors.

Specific activities of enzymes anabolizing and catabolizing PRPP. The specific activities of several enzymes were determined 2 hr post FUra or saline treatment i.p. of mice bearing colonic adenocarcinoma lines. Control PRPP synthetase activity in tumors 26 and 38 varied, with mean (± S.D.) or 6.7 ± 2.3 and 5.1 ± 1.4 nmoles·(mg protein)$^{-1}$·hr^{-1} respectively (Table 2). Treatment with 200 mg/kg FUra i.p. did not alter the specific activity of PRPP synthetase (Table 1). Moreover, tumors 51 and 11A demonstrated significantly lower specific activities of PRPP synthetase when saline and FUra treated, compared with the most sensitive of tumors, colon 26 ($P < 0.005$).

Survey of pertinent PRPP-utilizing enzymes demonstrated that, following administration of FUra (200 mg/kg, i.p.) in FUra-sensitive tumors (26 and 38), OPRTase activity was increased 158 and 37% over control respectively; a similar treatment of FUra-resistant tumor 51 was associated with an increase in OPRTase activity of 14% over baseline. In column 11A, OPRTase activity did not show any increase following treatment with FUra.

DISCUSSION

The present study demonstrates that the basal intratumoral PRPP levels in four murine colonic adenocarcinomas correlated with known antitumor activity of FUra in these tumors. Cadman *et al.* [11] have demonstrated recently that, in L1210 leukemia, the increased antitumor activity of FUra might be related to a 3- to 5-fold increase in intracellular PRPP levels as a consequence of pretreating the leukemic cells with methotrexate. Since intracellular PRPP levels play an important role in the mechanisms of the activation of FUra [24], and since PRPP K_m for the catabolizing enzymes and, in particular, OPRTase is 1.5–3.7 × 10^{-5} M [25], and the concentration of PRPP in the most FUra-sensitive tumor is approximately 8 μM, PRPP concentration, rather than enzyme concentration, may be rate-limiting for FUra metabolism in the intact cells [26].

Figure 1 demonstrates that 2 hr following administration of FUra i.p. (200 mg/kg) there is a substantial decrease in PRPP pools in the sensitive tumors, notably colonic tumor 26; however, the FUra-resistant tumors demonstrate no decrease in the PRPP pool following FUra administration. This latter phenomenon clearly suggests that PRPP was being utilized in the anabolism of FUra in the FUra-sensitive tumors. PRPP levels in the FUra-sensitive tumors followed a slow restitution and by 24 hr they had reached the baseline levels. Interestingly, FUra-resistant tumors demonstrated a minor increase in PRPP pool size at 24 hr compared with those of basal levels ($P > 0.2$). This latter phenomenon cannot be explained; however, if this finding is reproducible in the other FUra-resistant tumors, it might be advantageous to treat FUra-resistant

Table 2. Colonic tumors sensitive and resistant to FUra*

Colon tumor	Treatment	Anabolism PRPP synthetase	Catabolism HGPRT	Catabolism APRT	Catabolism OPRT
		← [nmoles·(mg protein)$^{-1}$·hr^{-1}] →			
26	Saline	6.7 ± 2.3	39.2 ± 10.5	35.1 ± 12.1	17.6 ± 8.5
	FUra	6.5 ± 1.8	37.1 ± 12.1	39.5 ± 18.3	45.4 ± 10.9
38	Saline	5.1 ± 1.4	48.7 ± 15.3	30.5 ± 14.8	18.5 ± 9.2
	FUra	3.8 ± 2.1	44.6 ± 18.1	31.8 ± 16.9	25.4 ± 10.5
51	Saline	2.1 ± 1.0	54.6 ± 17.6	8.6 ± 3.9	21.2 ± 4.9
	FUra	1.6 ± 0.9	64.5 ± 20.5	9.7 ± 4.5	24.3 ± 7.5
11A	Saline	2.8 ± 1.4	36.9 ± 12.7	31.5 ± 20.1	22.3 ± 8.8
	FUra	2.4 ± 1.5	46.8 ± 19.5	30.5 ± 18.9	15.8 ± 9.4

*Mice bearing subcutaneous colonic tumors were administered 200 mg/kg of FUra or saline i.p. Two hours later, the animals were killed, and tumors were removed and homogenized in 0.01 M Tris–HCl containing 0.15 mM EDTA and 0.5 mM dithiothreitol (pH 7.4). The homogenates were centrifuged at 12,000 *g* for 5 min and the cytosol fractions were kept prior to enzymic assays.

tumors at the time that PRPP pool sizes have increased, i.e. at 24 hr.

To determine the role of PRPP in the anabolism of FUra in murine adenocarcinoma lines under study, several anabolizing and catabolizing enzymes of PRPP were studied. In the FUra-sensitive line 26, specific activity of PRPP synthetase was notably higher than in FUra-resistant tumor lines 51 and 11A ($P < 0.005$). Moreover, the increase in the specific activity of PRPP synthetase was correlated with the degree of sensitivity and resistance of the colonic tumors to FUra ($P < 0.005$). Of the PRPP-utilizing enzymes examined in the FUra-sensitive tumors, only OPRTase was increased significantly ($P < 0.005$); there was no such correlation in FUra-resistant tumors ($P < 0.5$).

Several factors may contribute to the mechanisms of sensitivity or resistance of colonic adenocarcinoma cells to FUra [27]. We have shown previously that the rate of generation and clearance of one of the active metabolites of FUra, namely FdUMP, plays a major role in the *in vivo* mechanisms of sensitivity or resistance of the colonic adenocarcinoma cells to FUra [3]. However, we had not examined the basal or the FUra-treated levels of intratumoral PRPP in a systematic way. Our present *in vivo* data in murine colonic adenocarcinoma tumors clearly indicate that the measurement of the basal levels or PRPP, the cellular kinetic of its clearance, and the determination of the specific activities of PRPP synthetase and orotate phosphoribosyl transferase would be important variables of prediction or murine colonic cancer response to FUra. The latter variables are more easily determined data and could be useful parameters in selection of patients who will be treated with FUra.

REFERENCES

1. R. Duschinsky and E. Pleven, *J. Am. chem. Soc.* **79**, 4559 (1957).
2. P. S. Schein, D. Kisner and J. G. Macdonald, *Cancer* **36**, 2418 (1975).
3. B. Ardalan, D. M. Buscaglia and P. S. Schein, *Biochem. Pharmac.* **27**, 2009 (1978).
4. P. Danneberg, R. Langenbach and C. Heidelberger, *Biochemistry* **13**, 926 (1974).
5. R. I. Glazer and A. L. Peale, *Molec. Pharmac.* **16**, 270 (1979).
6. C. Heidelberger, N. K. Chaudhuri, P. Danneberg, D. Mooren, L. Greisbach, R. Duschinsky, R. J. Schnitzer, E. Pleven and J. Scheiner, *Nature, Lond.* **179**, 663 (1957).
7. D. Kessel, T. C. Hall and I. Wodinsky, *Science* **154**, 911 (1966).
8. P. Klubes, K. Connelly, I. Cerna and H. G. Mandel, *Cancer Res.* **38**, 2325 (1978).
9. P. Reichard, O. Skold and G. Klein, *Nature, Lond.* **183**, 939 (1959).
10. B. Ardalan, D. A. Cooney, H. N. Jayaram, C. K. Carrico, R. I. Glazer, J. S. Macdonald and P. S. Schein, *Cancer Res.* **40**, 1431 (1980).
11. E. Cadman, R. Heimer and L. Davis, *Science* **205**, 1135 (1979).
12. C. K. Carrico and R. I. Glazer, *Cancer Res.* **39**, 3694 (1979).
13. R. I. Glazer and M. Legraverend, *Molec. Pharmac.* **17**, 279 (1980).
14. R. J. Langenbach, P. V. Danneberg and C. Heidelberger, *Biochem. biophys. Res. Commum.* **48**, 1565 (1972).
15. D. S. Wilkinson, T. D. Tisty and R. J. Hanas, *Cancer Res.* **35**, 3014 (1975).
16. D. V. Santi, C. S. McHenry and H. Sommer, *Biochemistry* **13**, 471 (1974).
17. A. C. Sartorelli and W. A. Creasey, *Cancer Res.* **27**, 2201 (1967).
18. C. E. Myers, R. Diasio, H. H. Eliot and B. A. Chabner, *Cancer Treat. Rep.* **3**, 175 (1976).
19. C. E. Myers, R. C. Young and B. A. Chabner, *J. clin. Invest.* **56**, 1231 (1975).
20. R. Sawyer, R. Nayak, S. Spiegelman and D. Martin, *Proc. Am. Ass. Cancer Res.* **20**, 263 (1979).
21. B. Ardalan, M. Arakawa, D. Villacoste, H. Jayaran and D. A. Cooney, *Biochem. Pharmac.* **31**, 1509 (1982).
22. T. H. Corbett, D. P. Griswold, Jr., B. J. Roberts, J. C. Peckham and F. M. Schabel, Jr., *Cancer Res.* **35**, 2434 (1975).
23. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
24. I. H. Fox and W. N. Kelly, *Ann. intern Med.* **74**, 424 (1971).
25. M. J. C. Holland, A. M. Dilorenzo, J. Dancis, M. E. Balis, T. F. Yu and R. P. Cox, *J. clin. Invest.* **57**, 1600 (1976).
26. J. D. Laskin, R. M. Evans, H. K. Slocum, D. Burke and M. T. Hakala, *Cancer Res.* **39**, 383 (1979).
27. K. Ikenaka, T. Shirasaka, S. Kitano and S. Fujii, *Gann* **70**, 353 (1979).