Effects of 5-Fluorouracil on 5-Fluorodeoxyuridine 5'-Monophosphate and 2-Deoxyuridine 5'-Monophosphate Pools, and DNA Synthesis in Solid Mouse L1210 and Rat Walker 256 Tumors

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ABSTRACT

We have examined some of the factors determining the action of 5-fluorouracil (FUra) against a responsive and nonresponsive rodent tumor. In mice bearing the FUra-responsive solid L1210 tumor, \([^3H]dUrd\) incorporation into tumor DNA ceased promptly after FUra (100 mg/kg i.p.), and recovery was delayed for more than 96 hr. Incorporation of \([^3H]dUrd\) into bone marrow and small intestine, which were also strongly inhibited after FUra, recovered well before that of tumor. The levels of acid-soluble 5-fluorodeoxyuridine 5'-monophosphate (FdUMP) behaved in a reciprocal manner to the rate of \([^3H]dUrd\) incorporation in the tumor, although in small intestine and bone marrow this relationship was not as evident. Appreciable levels of FdUMP persisted in L1210 tumor for up to 72 hr after FUra. The 2-deoxyuridine 5'-monophosphate pool in the tumor was increased approximately 2-fold.

In rats bearing the relatively FUra-unresponsive solid Walker 256 carcinosarcoma, the incorporation of \([^3H]dUrd\) into DNA also promptly ceased after FUra (120 mg/kg s.c.), but then it rapidly resumed its normal rate within 48 hr. The effects of FUra on \([^3H]dUrd\) incorporation in rat bone marrow and duodenum were similar to those in the tumor except that recovery of \([^3H]dUrd\) incorporation was somewhat slower than that in the tumor. The levels of acid-soluble FdUMP increased and then decreased but not in precise association with inhibition and recovery of \([^3H]dUrd\) incorporation, respectively, in all tissues studied. Although initial levels of FdUMP in tumor were high, the anabolite was no longer measurable after 48 hr. The 2-deoxyuridine 5'-monophosphate pool in tumor was increased approximately 2-fold.

The prolonged inhibition of \([^3H]dUrd\) incorporation into DNA in the responsive L1210 tumor and the more rapid recovery in the relatively insensitive Walker 256 tumor correlated with the persistence of FdUMP in the L1210 tumor and its rapid disappearance in the Walker 256 tumor, but peak levels of FdUMP did not relate to tumor susceptibility to FUra.

INTRODUCTION

FUra\(^a\) has been widely used alone and in combination in the treatment of various solid tumors (8). The pharmacology of FUra has been described (8, 17). The major growth-inhibitory effect of FUra has been associated with the anabolite FdUMP, which, in the presence of 5,10-methenyltetrahydrofolate, binds covalently to thymidylate synthetase and thereby blocks DNA synthesis (15, 21). In addition, studies in various species have shown that FUra in the form of 5-fluorouridine 5'-monophosphate is incorporated into mRNA, rRNA, and tRNA and also interferes with rRNA maturation (8, 16, 24, 26).

For a proper understanding of the action of an anticancer drug in vivo, it is important to be able to relate the onset, duration, and recovery of inhibition to the concentration of the drug or its active metabolite(s) in tumor and sensitive tissues. A drug acting against a responsive tumor would be expected to produce a differential effect on a target, such as DNA synthesis, and possibly might be concentrated selectively in tumor compared to small intestine and bone marrow. Presumably, in a nonresponsive tumor such differential effects would not be seen.

Attempts to relate the action of FUra on DNA synthesis in tumor and sensitive tissues to concentration of FdUMP have been hampered by the difficulty in separating and detecting relatively small amounts of this active metabolite within the total FUra-nucleotide pool. Chadwick and Rogers (5), using \(^{14}C\)FUra in mice bearing solid L1210 tumor, demonstrated the selective persistence of FdUMP in tumor for up to 72 hr, whereas its concentration had declined in other tissues. Myers et al. (18, 20) developed a rapid and sensitive spectrophotometric assay for FdUMP and dUMP with thymidylate synthetase from Lactobacillus casei. In mice bearing the FUra-responsive P1534 ascites tumor, these authors (18, 20) found a persistence of FdUMP and preferential inhibition of DNA synthesis in tumor compared to bone marrow and duodenal mucosa after FUra. In a related study by Myers et al. (19), FUra produced more prolonged inhibition of DNA synthesis in tumor versus normal proliferating host tissues in mice bearing the FUra-responsive P1534 tumor but not in the less sensitive Ehrlich ascites tumor; however, tumor and tissue levels of FdUMP were not simultaneously determined.

The study was intended to compare the FdUMP and dUMP pools and the pattern of inhibition and recovery of DNA synthesis (as measured by incorporation of \([^3H]dUrd\) into DNA) in tumor, small intestine, and bone marrow in the FUra-responsive solid L1210 tumor in mice and the relatively FUra-unresponsive solid Walker 256 tumors in rats. It was anticipated that this approach would reveal factors that determine the action of FUra against a responsive and nonresponsive tumor. A preliminary report of our investigation has already appeared (12).

\(^a\)This work was supported by Grants CA 02978 and CA 17601, awarded by the National Cancer Institute, Department of Health, Education, and Welfare, Bethesda, Md., and by American Cancer Society Grant CII-110.

The abbreviations used are: FUra, 5-fluorouracil; FdUMP, 5-fluorodeoxyuridine 5'-monophosphate; dUrd, deoxyuridine; TCA, trichloroacetic acid.

Received February 2, 1978; accepted May 8, 1978.
MATERIALS AND METHODS

Chemicals. [6-3H]dUrd (28 Ci/mol) was obtained from New England Nuclear, Boston, Mass. FUra was obtained as a gift from Hoffmann-LaRoche Inc., Nutley, N. J. FdUMP, dUMP, and tetrahydrofolate were obtained from Sigma Chemical Co., St. Louis, Mo. Thymidylate synthetase (EC 2.1.1.45), prepared from dichloromethotrexate-resistant L. casei by the method of Crusberg et al. (6), was obtained from New England Enzyme Center, Tufts University Medical School, Boston, Mass. The enzyme formed 5.5 mmol dUMP per hr per mg protein at pH 7.0 and 30°. Dowex 1-X8 chloride anion-exchange resin, 200 to 400 mesh, was obtained from J. T. Baker Chemical Co., Phillipsburg, N. J. ScintiVerse (Triton-xylene base) liquid scintillation cocktail was obtained from Fisher Scientific Co., Fairlawn, N. J.

Animals and Tumors. All rats and mice were obtained from Sprague-Dawley Laboratories, Madison, Wis. The Walker 256 carcinosarcoma in the ascites form was maintained in male rats weighing 70 to 80 g. The L1210 lymphocytic leukemia in the ascites form was maintained in male DBA/2 mice weighing 18 to 20 g. Animals were caged in an air-conditioned room lighted from 8 a.m. to 8 p.m. and had free access to a standard Purina laboratory chow diet and tap water. Solutions of FUra for injection into animals were prepared in 1% Na2CO3 just prior to use. The solutions contained FUra in amounts appropriate to administer either 0.005 or 0.01 ml/g of rat or mouse body weight, respectively.

Effect of FUra on Weight of Solid Walker 256 Carcinosarcoma. Male rats weighing 150 to 160 g were given s.c. injections of 2 × 10⁸ Walker 256 ascites cells into the right thigh and on Day 5 were treated with FUra (120 mg/kg s.c.). The length and the width of tumors were determined daily by external caliper measurement and weight calculated according to the formula:

\[ \text{weight (mg)} = \frac{a \times b^2}{2} \]

where \( a \) = length (mm) and \( b \) = width (mm), based on the assumption that tumors were prolate spheroids with a density of 1.0 (22).

Effect of FUra on Survival of Mice Bearing Solid L1210 Tumors. Male C57BL/6 × DBA/2 F₁ (hereafter called B6D2F,) (23) mice weighing 18 to 20 g were given s.c. injections of 2 × 10⁶ L1210 ascites cells into the right axilla and on Day 6, when tumor weighed approximately 4 g, were treated with FUra (100 mg/kg i.p.). At intervals following drug administration, 6 mice/group at each time point received 50 μCi of [3H]dUrd (carrier free; 28 Ci/mmol). Twenty min after administration of [3H]dUrd (at which time the incorporation of label into DNA of tumor, small intestine, and bone marrow was still linear with respect to time), the mice were killed, the entire tumor was removed, and tumors from 3 mice were pooled for analysis. A 5-cm section of the small intestine immediately distal to the pylorus was removed and used for analysis. Bone marrow cells were flushed from 2 hind leg femora with cold 1 M acetic acid, and the solutions from a total of 4 femora from 2 rats were pooled for analysis.

For the mouse experiments, male B6D2F, mice weighing 18 to 20 g were given injections of 1 × 10⁶ L1210 ascites cells into the right axilla and on Day 6, when tumor weighed approximately 0.3 g, were treated with FUra (100 mg/kg i.p.). At intervals following drug administration, 6 mice/group at each time point received 50 μCi of [3H]dUrd (carrier free; 28 Ci/mmol). Twenty min after administration of [3H]dUrd (at which time the incorporation of label into DNA of tumor, small intestine, and bone marrow was still linear with respect to time), the mice were killed, the entire tumor was removed, and tumors from 3 mice were pooled for analysis. A 5-cm section of the small intestine immediately distal to the pylorus was removed, and sections from 3 mice were pooled and used for analysis. Bone marrow cells were flushed from 2 hind leg femora with cold 1 M acetic acid, and solutions from a total of 12 femora from 6 mice were pooled and used for analysis.

Tumor, small intestine, or bone marrow cells were extracted with 1 M acetic acid as previously described (18, 20) and saved for analysis of FdUMP and dUMP. The 1 M acetic acid-insoluble precipitate of each tissue was suspended in 1% Na2CO3 and centrifuged and the supernatant was decanted. The precipitate was washed 2 times with cold 10% TCA, defatted with ethanol and ether, incubated at 37° for 1 hr in 0.5 N KOH, and acidified with 50% TCA; the precipitate was washed 2 times with cold 10% TCA and then extracted twice with 5% TCA at 90°C. The DNA content of a 0.5-ml aliquot of the extract was determined by the diphenylamine method (4) and analyzed for radioactivity in ScintiVerse in a Beckman 250-DPM liquid scintillation counter. Results were corrected for counting efficiency with an external standard and were expressed as dpm/mg DNA. For comparison the incorporation of [3H]dUrd into DNA of untreated tumor-bearing rats or mice was measured on Day 5 or 6, respectively, after tumor inoculation. In untreated Day 5 tumor-bearing rats, the incorporation of [3H]dUrd into DNA of Walker 256 tumor, small intestine, and bone marrow was 65.2, 85.7, and 65.2 × 10⁵ dpm/mg DNA, respectively. Comparable figures on Day 6 for untreated mouse L1210 tumor, small intestine, and bone marrow were 3.43, 4.02, and 1.88 × 10⁶ dpm/mg DNA, respectively.

Measurement of Intracellular FdUMP and dUMP Pools following FUra in Vivo. The 1 M acetic acid extracts were lyophilized to remove acetic acid, and the residues were taken up in 2 ml of 50 mM Tris-10 mM 2-mercaptoethanol-1 mM disodium EDTA buffer, pH 7.4. An occasional sample of an extract from small intestine or bone marrow of rat or mouse produced interference in the spectrophotometric enzyme assay, resulting in an apparent stimulation or inhibition of the reaction. For removal of interfering substances, the reconstituted extract of either tumor, small intestine, or bone marrow was routinely subjected to chro-
matography on a Dowex 1-X8 chloride anion-exchange column. The Dowex 1-X8 chloride was prepared by washing with successive portions of 1 N NaOH, water, 3 N HCl, and water, and a 0.5- x 4-cm column was then prepared. The extract in 2 ml buffer was loaded onto the column and washed with 5 ml water; FdUMP and dUMP were eluted with 10 ml 0.05 N HCl, the eluant was lyophilized, and the residue was reconstituted in 2 ml of the Tris-2-mercaptoethanol-disodium EDTA buffer. Aliquots of the solution were assayed for FdUMP and dUMP with L. casei thymidylate synthetase by monitoring the generation of dihydrofolate as the increase in absorbance at 340 nm in a Beckman Acta II spectrophotometer at 37° as previously described (18, 20). Preliminary experiments showed that the recovery of FdUMP and dUMP added to tumor or tissues was 85 and 95%, respectively. Standard curves were linear within the ranges of 1 to 8 pmol FdUMP and 10 to 75 nmol dUMP.

RESULTS

Effect of FUra on Survival of Mice Bearing Solid L1210 Tumor. A single injection of FUra (50 to 200 mg/kg i.p.) on Day 6 after tumor inoculation resulted in an increase in median survival time of 18 to 47% compared to untreated controls (Table 1). The responsiveness of the solid L1210 tumor to FUra has been previously reported (11).

Relationship between Intracellular FdUMP and dUMP Pools and Rate of Incorporation of [3H]dUrd into DNA of Mouse L1210 Tumor, Small Intestine, and Bone Marrow following FUra. The levels of free FdUMP and dUMP and incorporation of [3H]dUrd in mouse solid L1210 tumor, small intestine, and bone marrow were monitored following administration of FUra (100 mg/kg i.p.). Two hr after FUra, [3H]dUrd incorporation into DNA of tumor (Chart 1A), small intestine (Chart 1B), and bone marrow (Chart 1C) was inhibited by 94 to 99%, compared to controls. The beginning of recovery of [3H]dUrd incorporation into DNA of small intestine and bone marrow was detectable at 24 hr and had returned to control rates in both of these tissues by 72 hr. In contrast, DNA synthesis in tumor, which was 25% of control at 72 hr, was still only 45% of control at 96 hr. Thus recovery of [3H]dUrd incorporation in tumor was selectively delayed, compared to that in normal host tissues. This differential effect produced by FUra on tumor DNA synthesis, compared to that on small intestine and bone marrow DNA synthesis, which we observed regularly, is consistent with the antitumor selectivity of FUra in this model system.

In the tumor (Chart 1A), acid-soluble FdUMP levels reached maximum values at 24 hr after FUra, gradually declined (48 and 72 hr), and eventually disappeared by 96 hr. Maximal inhibition of [3H]dUrd incorporation in general coincided with high levels of FdUMP (2 to 48 hr). The delayed recovery of [3H]dUrd incorporation, beginning at 72 hr, coincided with FdUMP concentrations below 10 pmol/mg tumor DNA. During this entire time (0 to 96 hr) the endogenous dUMP pool size fluctuated extensively and eventually expanded approximately 2-fold over control.

In the small intestine (Chart 1B) there was a considerable discrepancy between the elevation of levels of FdUMP and the inhibition of [3H]dUrd incorporation into DNA. The rate of DNA synthesis returned to normal between 48 and 72 hr.

<table>
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<th>FUra (mg/kg)</th>
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<sup>a</sup> p < 0.001 compared to control as determined by x² analysis.

Table 1

Effect of FUra administration on the survival times of B6D2F, mice bearing solid L1210 tumor

Male B6D2F, mice (18 to 20 g; 10 animals/group) were treated with a single i.p. injection of FUra on Day 6 after tumor inoculation. Control animals received an equivalent volume of 1% Na₂CO₃.

Chart 1. Relationship between intracellular FdUMP, dUMP, and incorporation of [3H]dUrd into DNA of mouse solid L1210 tumor (A), small intestine (B), and bone marrow (C). On Day 6 after tumor inoculation, mice were treated with FUra (FU) (100 mg/kg i.p.). Following a pulse with [3H]dUrd, the animals were sacrificed at the time intervals indicated, and the tissues were harvested and analyzed for DNA, radioactivity, FdUMP, and dUMP as described in "Materials and Methods." The combined results of 2 experiments are shown. Results are expressed as the percentage of [3H]dUrd incorporation ± S.E. (dpm/mg DNA; bar) of zero time controls, FdUMP (pmol/mg DNA), and dUMP (nmol/mg DNA).
after FUra, at which time appreciable amounts of acid-soluble FdUMP were still present. During this entire time the dUMP pool slowly declined, reaching a level of about 50% of control at 72 hr. Thus recovery of DNA synthesis occurred without any expansion of the dUMP pool.

In the bone marrow (Chart 1C) as in the small intestine, although levels of acid-soluble FdUMP were high when \[^{3}H\]dUrd incorporation into DNA was maximally inhibited, the correlation was poor and the rate of \[^{3}H\]dUrd incorporation into DNA of bone marrow and small intestine returned to normal by 72 hr, whereas appreciable amounts of FdUMP were still present. The acid-soluble FdUMP pool, which had begun to decline by 72 hr, disappeared completely by 96 hr. The highest concentrations of FdUMP were observed in bone marrow, compared to those in tumor and small intestine. The dUMP pool was essentially unchanged during the entire course (0 to 72 hr) of inhibition and recovery of DNA synthesis.

**Lack of Effect of FUra on Growth of Rat Solid Walker 256 Carcinoma.** A single injection of FUra (120 mg/kg s.c.) on Day 5 after tumor inoculation did not affect the growth of the solid Walker 256 tumor in that there were no significant differences in tumor weights on Days 6 to 12 between control and FUra-treated groups (Chart 2). In preliminary experiments we found that a single injection of FUra (120 mg/kg s.c.) was the maximally tolerated dose since higher doses of FUra caused early deaths that were apparently due to drug-related toxicity. Although not shown, a single injection of FUra (120 mg/kg s.c.) on Day 1 after tumor inoculation also failed to inhibit tumor growth. A slight response of a 1-day-old solid Walker 256 tumor to 7 successive daily doses of FUra (25 mg/kg) has been reported (25).

**Relationship between Intracellular FdUMP and dUMP Pools and Rate of Incorporation of \[^{3}H\]dUrd into DNA of Rat Walker 256 Tumor, Small Intestine, and Bone Marrow following FUra.** The levels of free FdUMP and dUMP and incorporation of \[^{3}H\]dUrd in rat solid Walker 256 tumor, small intestine, and bone marrow were monitored following administration of FUra (120 mg/kg s.c.). As can be seen, within 2 hr after FUra, \[^{3}H\]dUrd incorporation into DNA of tumor (Chart 3A) was inhibited by >98%, compared to controls. Recovery of \[^{3}H\]dUrd incorporation in tumor began within 24 hr, and the rate of incorporation had returned to control values by 48 hr. Initially, the effect of FUra on \[^{3}H\]dUrd incorporation in small intestine and bone marrow were similar to that of tumor (Chart 3, B and C, respectively). However, recovery of \[^{3}H\]dUrd incorporation in normal host tissues was delayed, compared to tumor, in that it was 72 hr before \[^{3}H\]dUrd incorporation in small intestine completely recovered. At that time the recovery in bone marrow had reached only 30% of control values. Therefore in rats bearing the FUra-unresponsive Walker 256 tumor, the differential effect produced by FUra on DNA synthesis in tumor versus normal tissues was reversed in that the tumor recovered before host normal tissues resumed their normal rate of DNA synthesis.

In the tumor (Chart 3A) the level of acid-soluble FdUMP was high 2 hr after FUra and then declined rapidly, so that it was undetectable by 48 hr. Maximal inhibition of \[^{3}H\]dUrd incorporation coincided with high levels of FdUMP at 2 hr, whereas the beginning of recovery of \[^{3}H\]dUrd incorporation at 24 hr and its eventual return to control levels by 48 hr were associated with the rapid decrease (24 hr) and eventual disappearance (48 hr) of FdUMP. During the inhibition and recovery of DNA synthesis, the dUMP pools had gradually expanded (at 72 hr) to approximately twice that of zero time levels, although these values were subject to considerable fluctuations.

In the small intestine (Chart 3B) the highest levels of FdUMP occurred 2 hr after FUra and coincided with extensive inhibition of \[^{3}H\]dUrd incorporation. Levels of FdUMP, which declined more slowly in comparison with those in tumor, were detectable up to 168 hr after FUra. Consistent with this slower decline of FdUMP, the recovery of incorporation of \[^{3}H\]dUrd into DNA of small intestine began after 24 hr and returned to control rate by 72 hr. At that time levels of FdUMP were still appreciable. The dUMP pool, which was found to be quite low in small intestine, increased >5-fold within 2 hr after FUra, remained elevated at 24 hr, and then rapidly declined to pretreatment levels by 48 hr.

In the rat bone marrow (Chart 3C), as was the case with tumor, high levels of FdUMP 2 hr after FUra coincided with marked inhibition of \[^{3}H\]dUrd incorporation. As had been observed for small intestine, peak levels of FdUMP were considerably less in bone marrow than in tumor. The concentration of acid-soluble FdUMP declined rapidly and was no longer detectable by 24 hr, whereas the recovery of the incorporation of \[^{3}H\]dUrd, which was evident at 48 hr,
FdUMP Persistence and Tumor Sensitivity to FUra

Chart 3. Relationship between intracellular FdUMP, dUMP, and incorporation of [3H]dUrd into DNA in rat solid Walker 256 tumor (A), small intestine (B), and bone marrow (C). On Day 5 after tumor inoculation, rats were treated with FUra (120 mg/kg s.c.). Following a pulse with [3H]dUrd, animals were sacrificed at the time intervals indicated, and the tissues were harvested and analyzed for DNA, radioactivity, FdUMP, and dUMP as described in "Materials and Methods." The combined results of 2 experiments are shown. Results are expressed as the percentage of [3H]dUrd incorporation ± S.E. (dpm/mg DNA; bar) of zero time control, FdUMP (pmol/mg DNA), and dUMP (nmol/mg DNA).

DISCUSSION

This study has demonstrated the basis, at least in part, for the contrasting actions of FUra against a responsive (L1210) and unresponsive (Walker 256) tumor. Within 2 hr after administration of FUra to either tumor-bearing rats or mice, the active anabolite FdUMP was rapidly formed and undoubtedly was responsible for the almost complete inhibition of the incorporation of [3H]dUrd into DNA of tumors and host normal tissues. In the L1210 tumor the initial peak concentrations of FdUMP slowly declined, and the persistence of FdUMP in this tumor for up to 72 hr after FUra administration paralleled the slow recovery of tumor DNA synthesis, which coincided with the eventual decline and disappearance of tumor FdUMP. In contrast, in both mouse small intestine and bone marrow, the incorporation of [3H]dUrd into DNA returned to control values despite the persistence of high levels of FdUMP. In the FUra-unresponsive Walker 256 tumor, the FdUMP pool, which reached extremely high levels soon after FUra administration, was depleted within 48 hr. This depletion of tumor FdUMP also coincided with the complete recovery of tumor DNA synthesis, which in fact preceded the recovery of DNA synthesis in bone marrow and small intestine. Similar to the Walker tumor, the recovery of DNA synthesis in these normal tissues coincided with either the decline (small intestine) or disappearance (bone marrow) of FdUMP.

The persistence of FdUMP in solid L1210 tumor, which we observed, is consistent with the previous reports of Chadwick and Rogers (5). These investigators monitored FdUMP and other FUra nucleotides in tumor and various other tissues by a combination of ion-exchange and thin-layer chromatography after administration of a single injection of [2-14C]FUra (200 mg/kg i.v.) to B6D2F1 mice bearing a 6-day solid L1210 tumor. After the administration of FUra, markedly higher concentrations of FdUMP were found in tumor at 72 hr, compared to various other tissues including bone marrow and small intestine.

In C6D2F1 mice bearing the FUra-responsive P1534 ascites tumor, Myers et al. (18, 20) found that FdUMP persisted in tumor as well as bone marrow and, to a lesser extent, duodenal mucosa for up to 7 days after a single i.p. injection of FUra (100 mg/kg). Consistent with the slow decline of FdUMP in the tumor was the preferential inhibition characterized by a delayed recovery of the incorporation of [3H]dUrd into tumor DNA compared to that in duodenal mucosa and bone marrow. The decline in tumor FdUMP levels coincided with a sustained expansion of the dUMP pool together with the recovery of DNA synthesis. In contrast to the report of Chadwick and Rogers (5), at 72 hr after FUra, neither our results nor those of Myers et al. (18, 20) showed as marked a specificity of retention of FdUMP in tumor compared to bone marrow or small intestine.

Myers et al. (19) also followed the effect of FUra on DNA synthesis of tumor, gastrointestinal mucosa, and bone marrow in Swiss mice bearing the relatively FUra-unresponsive Ehrlich ascites tumor. The effects of FUra on DNA synthesis were characterized by an initial inhibition followed by a rapid and nonselective recovery of tumor and host normal tissues. Tumor and normal organ levels of FdUMP were not determined but, presumably, they were sufficient to account for the early inhibition of DNA synthesis. FdUMP formed from FUra has been detected in Ehrlich ascites cells in vitro (2) and in vivo (7) 1 hr after FUra administration. The contrasting effect of FUra against DNA synthesis in the Ehrlich and P1534 tumors, as described by Myers et al. (18, 19), is confirmed by our results with the FUra-responsive L1210 tumor and FUra-unresponsive
Walker tumor.

Myers et al. (18), using C6D2F1, mice bearing P1534 ascites cells, found that FUra treatment resulted in a consistent expansion of the dUMP pool in tumor, gastrointestinal mucosa, and bone marrow. For example, following a dose of FUra (100 mg/kg), the dUMP pool in tumor, gastrointestinal mucosa, and bone marrow showed a maximum expansion to 6, 4, and 21 times control level, respectively. Myers et al. (18) attributed the recovery of thymidylate synthetase (and thus DNA synthesis) to both a fall in intracellular levels of FdUMP and a progressive accumulation of the competitive substrate dUMP, the latter of which protects newly synthesized thymidylate synthetase from inactivation by FdUMP. In contrast, in our studies with B6D2F1 mice bearing L1210 solid tumor treated with FUra (100 mg/kg i.p.), we did not find such a pronounced expansion in dUMP pools that, at maximum in tumor, small intestine, or bone marrow, were 2, 1.2, and 2 times that of control, respectively. However, in Sprague-Dawley rats bearing solid Walker 256 tumor treated with FUra (120 mg/kg s.c.), the dUMP pools in tumor, small intestine, and bone marrow expanded at maximum to 5, 7, and 4 times that of control, respectively. Thus expansion of dUMP pools in Sprague-Dawley rats may contribute to rapid recovery of tissues from FdUMP inhibition, particularly in the Walker 256 tumor in which the endogenous pool of dUMP is 65-fold greater than that in the mouse L1210 tumor.

In the monitoring of DNA synthesis by the incorporation of [3H]dUrd into DNA, Myers et al. (18, 19) used a correction factor to calculate the specific activity of DNA. This correction factor took into account the consistent expansion in dUMP pool sizes, which Myers et al. (18, 19) observed in all tissues following FUra administration. In contrast to Myers et al. (18, 19), we observed rather erratic changes in the endogenous dUMP pool sizes. In addition we found only small changes in the dUMP pool sizes in the mouse, whereas these changes were somewhat larger in the rat. For these reasons our measurements of DNA specific activity as reported were done without calculating for changes in the nucleotide pool sizes. Although not shown, when the changes in dUMP pool sizes were taken into account, they did not alter appreciably the temporal relationships between inhibition and recovery of DNA synthesis in tumor and host normal tissues in our models.

Additional factors that must be taken into consideration in any in vivo study involving [3H]dUrd incorporation into DNA are the differences in the activities of uridine phosphorylase (EC 2.4.2.3, uridine:orthophosphate ribosyltransferase), thymidine phosphorylase (EC 2.4.2.4, thymidine:orthophosphate deoxyribosyltransferase), and thymidine kinase (EC 2.7.1.75, ATP:thymidine 5'-phosphotransferase) in different animals as well as in different tissues of the same animal (9, 13, 14, 27, 28). These enzymes, which can metabolize [3H]dUrd, will in part determine the utilization of [3H]dUrd for incorporation into DNA of tumor and normal tissues. Therefore our results cannot exclude the possibility that alterations after FUra treatment in the activities of any of these 3 enzymes in different tissues may contribute to the differential effects on the incorporation of [3H]dUrd into DNA, which we have observed.

Since the Walker 256 tumor is unresponsive to FUra despite the formation of high levels of FdUMP, other factors must contribute to or be responsible for the lack of therapeutic response. One possible mechanism of resistance by tumor to FUra, as well as other pyrimidine and purine analogs, is a failure to anabolize the analog to the nucleotide level (3, 8). Clearly, this is not the case for the Walker 256 tumor, which actually had higher levels of FdUMP initially than did the more responsive L1210 tumor. The enzymatic assay method for FdUMP of Myers et al. (18, 20), which we used, as well as the chromatographic method of Chadwick and Rogers (5), measure only free acid-soluble FdUMP since the covalent complex formed from thymidylate synthetase, FdUMP, and 5,10-methylenetetrahydrofolate is not acid extractable (15, 18, 20, 21). Thus the total tissue content of FdUMP is not determined. At the present time the basis for the unresponsiveness of the Walker tumor to FUra, characterized by the rapid disappearance of FdUMP and the early recovery of DNA synthesis, is unknown. Other possibilities that may be responsible for the FUra insensitivity include high levels of thymidylate synthetase; increased turnover of this enzyme; decreased affinity of complex formation between enzyme, FdUMP, and 5,10-methylenetetrahydrofolate, altered levels of 5,10-methylenetetrahydrofolate; increased rate of dephosphorylation of FdUMP (e.g., by phosphatases); or enhanced protection by dUMP, in the presence of FdUMP, of newly synthesized thymidylate synthetase.

The disappearance of acid-soluble FdUMP in a given tissue is not always a reliable indicator of the return of DNA synthesis to its normal rate. For example, both our results and those of Myers et al. (18, 20) showed that complete recovery of DNA synthesis occurred in mouse bone marrow and small intestine in the presence of appreciable levels of FdUMP. On the other hand we found that in rat bone marrow the disappearance of FdUMP preceded the complete recovery of DNA synthesis. At this time it appears that much more remains to be elucidated with respect to the relationship between intracellular levels of free and enzyme-bound FdUMP, thymidylate synthetase, 5,10-methylene tetrahydrofolate, dUMP, and DNA synthesis in both FUra-responsive and -unresponsive tumors before all of the factors determining the basis for the antitumor selectivity of FUra are completely understood.

In a series of rodent tumors, a correlation has been demonstrated between conversion of FUra to nucleotides and enhanced survival following FUra treatment (10). Furthermore, Ardalan et al. (1) showed that the sensitivity of individual mouse colonic tumors correlated with the tumor levels of FdUMP measured 2 hr after FUra administration. However, our results comparing the absolute levels of FdUMP in the solid L1210 and Walker 256 tumors at 2 hr after drug administration do not agree with this correlation. Based on our studies with solid L1210 and Walker 256 tumors and on that of others with solid L1210 (5) and P1534 ascites tumor (18, 20), it appears that the persistence of FdUMP in tumor correlated best with the therapeutic effect observed. This hypothesis is subject to critical analysis in a carefully monitored series of patients on FUra therapy for tumors for which serial biopsy sampling is possible so that retention of FdUMP can be assayed in relation to tumor response to FUra. Such an assay may also then be useful as...
a predictor of responsiveness of human solid tumors to FUra.

REFERENCES


Effects of 5-Fluorouracil on 5-Fluorodeoxyuridine 5’-Monophosphate and 2-Deoxyuridine 5’-Monophosphate Pools, and DNA Synthesis in Solid Mouse L1210 and Rat Walker 256 Tumors

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