Regulation of the Activation of Fluorodeoxyuridine by Substrate Competition and Feedback Inhibition in 647V Cells

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ABSTRACT

Fluorodeoxyuridine (FdUrd) is a cytotoxic analogue of thymidine which requires activation by thymidine kinase to FdUMP. FdUMP inhibits thymidylate synthetase and, thus, the synthesis of dTTP. 5'-Aminothymidine (5'-AdThd) can antagonize the feedback inhibition exerted by dTTP on thymidine kinase activity and thereby stimulate FdUrd phosphorylation. This provided a novel approach to assess the degree to which end product inhibition regulates the phosphorylation of FdUrd. We used 5'-AdThd to investigate the effects of dThd and IdUrd on the regulation of FdUrd uptake in intact 647V cells, a human bladder cancer cell line. Contributions from catabolic processes were found not to be important in our system. We detected no nucleoside phosphorylase activity in the 647V cells or any effect of 5'-AdThd on the breakdown of 5-fluorodeoxyuridine monophosphate to FdUrd by crude preparations from these cells. Thus, phosphorylation by thymidine kinase determined FdUrd uptake (phosphorylation). In the absence of added nucleosides the rate of FdUrd uptake increased in a time dependent fashion. Diminished feedback inhibition of thymidine kinase appeared to be an important factor, as evidenced by a decrease in intracellular dTTP pools and a time dependent loss in the ability of 5'-AdThd to stimulate FdUrd uptake. Thymidine and iododeoxyuridine inhibited FdUrd phosphorylation (uptake) by two mechanisms: competition for the active site of thymidine kinase and increased feedback inhibition. Increased feedback inhibition was indicated by stimulation of FdUrd uptake by 5'-AdThd. The effects of IdUrd on FdUrd uptake were also time dependent, presumably reflecting accumulation of iododeoxyuridine triphosphate and dTTP pools. FdUrd cytotoxicity was modulated by dThd, IdUrd, and 5'-AdThd in parallel to their perturbation of FdUrd uptake. Individually they reduced the growth inhibitory properties of FdUrd. These results show that the regulation of FdUrd uptake is critically dependent on the presence of dThd and IdUrd and emphasize the potential importance of circulating levels of these nucleosides in mediating FdUrd activation and cytoxicity.

INTRODUCTION

FdUrd is phosphorylated by thymidine kinase to FdUMP (2), a potent inhibitor of thymidylate synthetase (3–7), resulting in blockade of the de novo formation of dTTP and, consequently, DNA synthesis (8–11). If other substrates for thymidine kinase such as dThd or its cytotoxic analogue IdUrd are present, the extent of FdUrd phosphorylation will depend, in part, on competition for the active site. In addition, thymidine kinase activity is regulated by feedback inhibition mediated by the end products of dThd and IdUrd salvage, dTTP and IdUTP, respectively (12–14). Therefore, the rate at which nucleosides are phosphorylated by thymidine kinase will depend on both substrate competition and feedback inhibition. The aim of this study was to determine the role of these factors in regulating FdUrd phosphorylation in 647V cells, a human bladder cancer cell line. This presents an interesting problem because: (a) the conversion of FdUrd to FdUMP results in a depletion of intracellular dTTP, a critical regulator of thymidine kinase activity; (b) FdUrd activation may be affected by circulating levels of dThd, either by substrate competition or by increasing feedback inhibition; and (c) the administration of IdUrd in combination with FdUrd, which can produce increased cytotoxicity (15–19), may inhibit FdUrd activation.

These points are illustrated in Fig. 1. Interactions at the level of substrate competition (bracket) and feedback inhibition (dashed lines) are depicted. The importance of interactions at the active site (substrate competition) will depend on the concentration of the alternate substrates and their affinity for thymidine kinase. The intracellular levels of feedback inhibitors can be increased by the salvage of dThd or IdUrd or decreased by inhibition of thymidylate synthetase induced by FdUMP. 5'-AdThd can antagonize the inhibitory effects of dTTP and IdUTP on thymidine kinase by a direct interaction with the enzyme (20–22). This unusual effect provides an experimental tool to assess the role of feedback inhibition since the effects of 5'-AdThd on thymidine kinase activity are critically dependent on the presence of feedback inhibitors. If there is sufficient inhibition, then 5'-AdThd can stimulate enzyme activity by diminishing the inhibition exerted at the regulatory site. In the absence of feedback inhibition or at high concentrations of 5'-AdThd, competitive interactions at the active site predominate (20–22). Since 5'-AdThd, a selective inhibitor of herpes simplex virus replication (23), is neither phosphorylated by the mammalian thymidine kinase nor cytotoxic (24, 25), then the effects of dThd and IdUrd on the regulation of FdUrd activation could be directly elucidated in intact cells using 5'-AdThd.

In 647V cells, dThd and IdUrd effectively inhibited the uptake of FdUrd by both substrate competition and, to a significant degree, feedback regulation. Furthermore, in growth inhibition experiments IdUrd, dThd, and 5'-AdThd independently reduced FdUrd cytotoxicity. These data accentuate the importance of the activation of FdUrd with regard to its cytotoxic properties and its possible modulation by circulating levels of dThd and IdUrd.

MATERIALS AND METHODS

Materials. 5'-AdThd and dThd were purchased from Sigma Chemical Co. (St. Louis, MO). FUra, FdUrd, FdUMP, IdUrd, and IdUMP were obtained from Calbiochem-Behring Co. (La Jolla, CA). dTTP and IdUTP were obtained from P. L. Biochemicals (Milwaukee, WI). [8-3H]dATP (20 Ci/mmol), [6-3H]IdUrd (13 Ci/mmol), [methyl-3H]dTd (70 Ci/mmol), [2-14C]FdUrd (56 mCi/mmol), [6-3H]FdUMP (20 Ci/mmol), and [6-3H]FdUrd (20 Ci/mmol) were purchased from Moravek Biochemicals, Inc. (Brea, CA). Alamine (tri-n-octylamine) was obtained from Aldrich Chemical Co. (Milwaukee, WI) and freon (1,1,2-trichloro-1,2,2-trifluoroethane) from J. T. Baker Chemical Co. (Phillipsburg, NJ). Unless otherwise specified, all other reagents were purchased from Sigma.

Cell Culture. The growth and maintenance of the human bladder
cells were negative for Mycoplasma using the DNA fluorochrome
and earlier (26) have been described previously (22). These
experiments in which further analysis by HPLC was required, the acid
were exposed to 3 mM [3H]FdUrd (1 ±Ci/ml) and to the test compounds
with 10% fetal bovine serum. After a 1-h incubation in minimal essential
formed (percentage of control) versus inhibitor concentration.
Different concentrations were used for each substrate. To estimate the
reciprocal plots of initial velocity versus substrate concentration. Four
Km values were determined from double-
experiments from 647V cells; (b) the uptake of FdUrd in intact cells;
and (c) FdUrd cytotoxicity. Our strategy included the use of 5'-
AdThd as an indicator of the degree of feedback inhibition of
thymidine kinase.

RESULTS
Since interactions at the active or the regulatory sites of
thymidine kinase can have significant consequences on FdUrd
activation, we determined the effects of dTdp and IdUrD on:
(a) the phosphorylation of FdUrd in purified enzyme prepara-
tions from 647V cells; (b) the uptake of FdUrd in intact cells;
and (c) FdUrd cytotoxicity. Our strategy included the use of 5'-
AdThd as an indicator of the degree of feedback inhibition of
thymidine kinase.

Modulation of FdUrd Phosphorylation by Purified Thymidine
Kinase. The relative binding affinities of the various compounds
for purified thymidine kinase were estimated by determining
Km and IC50 values (Table 1). The Km values for dTdp and
IdUrD, approximately 2 μM, were about 4-fold lower than that
for FdUrd. 5'-AdThd and dTTP had high affinity for thymidine
kinase and had IC50 values of about 2 μM.
Our experimental strategy, involving the use of 5'-AdThd as an
indicator of the degree of feedback inhibition of thymidine kinase in situ, was based on the observation that 5'-AdThd can
antagonize feedback inhibition exerted by dTTP when dTdp is
used as the substrate (20, 22). Since the affinity of thymidine
kinase was found to be lower for FdUrd than for dTdp it was
important to determine if 5'-AdThd could antagonize the ef-
effects of dTTP on enzyme activity when FdUrd was used as the
substrate. The effects of 5'-AdThd on the phosphorylation of
FdUrd in the presence and absence of dTTP are shown in Fig.
2. In the absence of dTTP, 5'-AdThd and dTTP had high affinity for thymidine
kinase and had IC50 values of about 2 μM.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Kinetic parameters for 647V thymidine kinase</th>
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<tr>
<td>Substrate</td>
<td>IC50 (μM)</td>
</tr>
<tr>
<td>dTdp</td>
<td>5.0</td>
</tr>
<tr>
<td>dUrd</td>
<td>3.0</td>
</tr>
<tr>
<td>FdUrd</td>
<td>11.0</td>
</tr>
<tr>
<td>5'-AdThd</td>
<td>1.8</td>
</tr>
<tr>
<td>dTTP</td>
<td>1.8</td>
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* IC50 values were determined from experiments with purified thymidine kinase using 3 μM [3H]FdUrd as the substrate.
Km values were determined from double reciprocal plots of the initial velocity versus concentration of the substrate.

Fig. 1. Biochemical interactions affecting thymidine kinase in the presence of
dTdp, IdUrD, 5'-AdThd, and FdUrd. See text for details. Ü, inhibition.
maximal stimulation of enzyme activity; (b) as the degree of enzyme activity decreased at higher concentrations of dTTP, the percentage of increase in thymidine kinase activity produced by 5'-AdThd was augmented; (c) as the concentration of 5'-AdThd was increased, inhibition of enzyme activity by interactions at the active site predominated and a biphasic dose-response curve was generated. For example, in the presence of 5 μM dTTP, 1 μM 5'-AdThd increased FdUrd phosphorylation 2-fold whereas in the presence of 10 μM dTTP 3 μM 5'-AdThd was necessary to increase thymidine kinase activity more than 10-fold. These findings demonstrated that the effect of 5'-AdThd on FdUrd phosphorylation, as had been seen with dThd or IdUrd as the substrate (20, 22), was critically dependent on feedback inhibition. Thus, 5'-AdThd could be used as an experimental tool to assess the degree to which feedback inhibition regulates the phosphorylation of FdUrd.

Metabolism of FdUrd in 647V Cells. Besides thymidine kinase, other enzymes of the pyrimidine metabolic pathway can metabolize FdUrd. Among these, nucleoside phosphorylase (35) and cytoplasmic 5'-nucleotidase (36) could potentially affect FdUrd uptake. The cleavage of FdUrd to FUra could limit the availability of substrate for thymidine kinase. Also, once phosphorylated by thymidine kinase, FdUMP could be degraded to FdUrd, which can then permeate the membrane. These reactions could decrease FdUrd uptake since quantitation is dependent on the intracellular retention of phosphorylated derivatives.

We found no evidence of the breakdown of FdUrd to FUra in 647V cells under our culture and experimental conditions (Table 2). No breakdown of dThd to thymine or IdUrd to iodouracil has been detected in this cell line either (data not shown). In crude preparations of cytoplasmic 5'-nucleotidase we found that the conversion of [3H]FdUMP to FdUrd was not affected by 5'-AdThd (Table 2). Furthermore, 5'-AdThd does not affect the breakdown of dTMP or IdUMP to dThd or IdUrd, respectively (data not shown). In this cell line we found that all the radioactivity retained in our uptake experiments was recovered as FdUrd and FdUMP, as determined by HPLC analysis (Table 2). Thus, the results obtained from the intact cell studies (see below) reflect interactions at the level of FdUrd phosphorylation.

Modulation of the Uptake of FdUrd in 647V Cells. If feedback inhibition normally regulates thymidine kinase in 647V cells, then the rate of FdUrd phosphorylation should increase in response to the depletion of dTTP pools produced by FdUMP (Fig. 1). As dTTP pools fall and feedback inhibition of thymidine kinase is diminished, the ability of 5'-AdThd to enhance FdUrd uptake should also decrease. In fact, this was seen. The uptake of 3 μM FdUrd was measured at 10, 20, and 30 min in cells incubated in media supplemented with dialyzed fetal bovine serum and various concentrations of 5'-AdThd (Fig. 3). In the absence of 5'-AdThd, the initial rate of FdUrd uptake increased between 10 and 30 min (Fig. 3A). In contrast, the stimulation of FdUrd uptake produced by 5'-AdThd decreased with time (Fig. 3). For example, 10 μM 5'-AdThd increased FdUrd uptake 164% of control at 10 min, but uptake was decreased to 92% of control at 30 min. Similarly, the inhibition of FdUrd uptake produced by higher concentrations of 5'-AdThd increased with time. FdUrd uptake was minimally affected by 30 μM 5'-AdThd at 10 min, but by 30 min it was only 50% of control. A significant reduction in dTTP pool sizes was observed after exposure to FdUrd (Table 3); however, the perturbation was not strictly time dependent. These results indicate that exposure to FdUrd produces a time dependent decrease in the degree to which thymidine kinase is feedback inhibited.

The use of FdUrd to potentiate the cytotoxic and radiosensitizing properties of IdUrd has been suggested as a strategy to improve therapy (15, 16). The success of this approach would depend, in part, on two important metabolic interactions: (a) competition for phosphorylation by thymidine kinase; and (b) their antithetical effects on total intracellular feedback inhibitor pools (Fig. 1). Thus, we examined the effects of IdUrd on FdUrd uptake. IdUrd prevented (Fig. 4A) the time dependent reduction in dTTP pools observed after exposure to FdUrd (Table 3); however, the perturbation was not strictly time dependent. These results indicate that exposure to FdUrd produces a time dependent decrease in the degree to which thymidine kinase is feedback inhibited.
The results obtained from the enzymatic assay for dTTP pools did not reflect large differences between the treatment of cells with FdUrd alone or in the presence of IdUrd, nevertheless, the decrease in dTTP pools was slower when IdUrd was present. HPLC analysis indicated that exposure to IdUrd resulted in the formation of dTTP (<1 pmol/10⁶ cells derived from the exogenous source) and IdUTP pools (about 6 pmol/10⁶ cells; Table 3). These data indicate that an increase in the feedback inhibition of thymidine kinase is an important component of the inhibition of FdUrd uptake produced by IdUrd.

The effect of IdUrd on FdUrd uptake demonstrated the sensitivity of FdUrd phosphorylation by thymidine kinase to feedback inhibition. This prompted us to also consider the effects of dThd on FdUrd uptake. dThd can be present in the plasma at concentrations as high as 0.8 µM (37). Thus, we evaluated the effect of 0.5 µM dThd on the uptake of FdUrd and its perturbation by 5'-AdThd. After a 1-h exposure, dThd inhibited the uptake of FdUrd from 640 to 180 pmol/10⁶ cells while 3 µM IdUrd resulted in a greater inhibition (20 pmol/10⁶ cells; Fig. 5). Again, two factors could account for these observations: substrate competition and feedback inhibition (Fig. 1).

We evaluated the contribution of substrate competition by assessing the effect of dThd on the phosphorylation of 3 µM FdUrd by purified preparations of thymidine kinase. We found that 0.5 µM dThd inhibited FdUMP formation by about 30% (Fig. 6, open circles). In intact cells, however, the same concentration of dThd inhibited the uptake of 3 µM FdUrd by about 60%. Thus, in addition to substrate competition, another factor likely contributed to the inhibition of FdUrd uptake.

The role of feedback inhibition was assessed by exposing the cells to various concentrations of 5'-AdThd. 5'-AdThd stimulated FdUrd uptake in the presence of 3 µM IdUrd or 0.5 µM dThd (Fig. 7). Two points are of interest: (a) while the maxim...
HPLC analysis indicated that in the presence of FdUrd 3 μM [3H]IdUrd provided about 11 ± 4 pmol/106 cells (mean ± SE, n = 8). These observations are consistent with a diminished degree of feedback inhibition of thymidine kinase in the FdUrd-treated cells.

Modulation of FdUrd Cytotoxicity. We did growth inhibition studies to investigate whether the perturbation of FdUrd activation influenced its cytotoxicity. The cells were exposed to 3 μM FdUrd, 3 μM IdUrd, 0.5 μM dThd, and 10 μM 5'-AdThd alone or in combinations for 1 h. The cells were washed with PBS and then incubated for 72 h in the standard media. We found that dThd, IdUrd, and 5'-AdThd substantially reduced the inhibition of cell growth induced by FdUrd (Table 5). For example, exposure to 3 μM FdUrd inhibited growth by 71%, while, in the presence of 0.5 μM dThd, 3 μM IdUrd or 10 μM 5'-AdThd, growth was inhibited by 38, 26, or 48%, respectively.

**DISCUSSION**

In this report we have described the interactions that affect FdUrd uptake in 647V cells. Under our experimental conditions FdUrd metabolism was primarily determined by thymidine kinase. In general, FdUrd uptake could be affected by: (a) nucleoside transport (38); (b) thymidine kinase (2); (c) nucleoside phosphorylase activity; and (d) FdUMP phosphatase activity. Nucleoside transport was not a major consideration in our study because: (a) our drug exposures were over relatively long time periods, considering that nucleoside transport equilibrates within seconds (45); and (b) we used nucleoside concentrations much lower than the Km for the high capacity nucleoside transporter (39).

The catabolic processes were not of importance in this study. We were unable to detect breakdown of FdUrd to FUra, a process mediated by a nucleoside phosphorylase (35). Furthermore, the breakdown of FdUMP to FdUrd, in crude preparations from 647V cells, was not affected by 5'-AdThd (Table 2). This process could be mediated by a cytoplasmic 5'-nucleotidase, yet this is an enzyme with a high Km (e.g., dTMP, 2 mM) and found to be highest in confluent cultures (40). Therefore, in logarithmically growing 647V cells, FdUrd uptake is predominantly affected by thymidine kinase activity.

We found that the regulation of FdUrd phosphorylation by thymidine kinase in intact cells was affected by two opposing components: (a) decreased feedback inhibition due to the FdUMP-induced depletion of dTTP and (b) increased feedback inhibition presumably due to the replenishment of feedback inhibitor pools (dTTP or IdUTP) by dThd or IdUrd salvage. In the absence of dThd or IdUrd, FdUrd uptake increased with time (Fig. 3) due to a diminished feedback inhibition of thymidine kinase. This was evidenced by the inhibitory role of 5'-AdThd on FdUrd uptake (Figs. 3 and 4) and by the depletion of intracellular dTTP pools (Table 3). Although we used relatively short time exposures to FdUrd, others have reported

<table>
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<th>Treatment</th>
<th>dTTP pools (pmol/106 cells)</th>
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<tr>
<td>-dThd</td>
<td>70 ± 4</td>
</tr>
<tr>
<td>FdUrd</td>
<td>25 ± 2</td>
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<tr>
<td>FdUrd + IdUrd</td>
<td>30 ± 1</td>
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<th>Exposure with 3 μM FdUrd</th>
<th>Cell growth (% of control)</th>
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<tr>
<td>-dThd</td>
<td>+0.5 μM dThd</td>
</tr>
<tr>
<td>Alone</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>10 μM 5'-AdThd</td>
<td>52 ± 8</td>
</tr>
<tr>
<td>3 μM IdUrd</td>
<td>74 ± 8</td>
</tr>
<tr>
<td>3 μM IdUrd + 10 μM 5'-AdThd</td>
<td>68 ± 8</td>
</tr>
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</table>
significant perturbations in dTTP pools under a similar time course. For example, Cheng and Nakayama (8) showed that exposure of HeLa cells to 0.1 μM FdUrd for 1 h reduced dTTP pools from 800 to 300 pmol/10^7 cells.

The presence of dThd or IdUrd inhibited FdUrd uptake in 647V cells (Figs. 4, 5, and 6). In their presence, feedback regulation became a determinant factor in modulating the phosphorylation of FdUrd as evidenced by the ability of 5'-AdThd to enhance FdUrd uptake (Figs. 4 and 7). The stimulatory effect of 5'-AdThd on the uptake of FdUrd is due to a direct interaction with thymidine kinase as seen in the purified enzyme studies (Fig. 2). An alternative explanation could be that IdUTP pools are not as elevated as in the absence of 5'-AdThd. However, this is not expected to be the case because: (a) 5'-AdThd enhances IdUrd uptake and cytotoxicity (and IdUTP is the toxic species); and (b) 5'-AdThd itself is very nontoxic, which is inconsistent with a role as an inhibitor of dTTP formation.

Using the DNA polymerase assay, an indirect measurement of intracellular dTTP, we observed less increases in dTTP and IdUTP pools induced by IdUrd in the FdUrd-treated cells than expected from the uptake experiments (Tables 3 and 4). HPLC analysis of the amount of dTTP or IdUTP provided by exogenous IdUrd showed moderate levels of IdUTP and low levels of dTTP contributed by this route (Tables 3 and 4).

We expected to observe a significant expansion of feedback inhibitor pools (dTTP, IdUTP) in the presence of IdUrd because: (a) FdUrd uptake was substantially enhanced by 5'-AdThd in the presence of IdUrd and the perturbation of thymidine kinase activity by 5'-AdThd is dependent on the degree of inhibition of the enzyme (Fig. 2); (b) in the presence of IdUrd less FdUMP was formed (Figs. 4 and 5) and thus less reduction of dTTP pools should result; and (c) IdUrd is a precursor to both dTTP and IdUTP. Although large expansions of feedback inhibitor pools were not observed, the FdUrd-induced depletion of dTTP pools was slower in the presence of IdUrd (Tables 3 and 4). The discrepancy between the expected perturbation of dTTP pools and their measurement can be explained in two ways: (a) the enzymatic assay is an indirect method for quantification and as such it is susceptible to errors (29); and (b) FdUMP inhibition of thymidylate synthetase could result in the accumulation of dUTP which could also serve as precursor for the assay (41) and thus confound the results. Also, in our hands, IdUTP is utilized less efficiently for the polymerase reaction than dTTP (data not shown). On the other hand, it is important to consider also that IdUTP is a more potent inhibitor of thymidine kinase than dTTP (14, 42). IdUTP is about 3 times more potent than dTTP and 100 times more potent than dUTP as an inhibitor of thymidine kinase purified from HCT 116, a human colon carcinoma cell line (42). Furthermore, dTTP pools could be compartmentalized (43), which could make the interpretation of total dTTP pool sizes more difficult. Therefore, absolute quantities of the feedback inhibitor pools do not necessarily provide a direct estimate of the degree of feedback regulation of the enzyme. For these reasons, the data from the enzymatic assay should be interpreted with caution.

Previous studies have shown that pretreatment of cells with high concentrations (100 μM) of dThd significantly reduce intracellular FdUMP pools after exposure to FdUrd (44, 45). In other studies, 10 μM dThd and 100 μM deoxyuridine inhibited the uptake of FdUrd in Ehrlich ascites tumor cells (46, 47). In such studies using relatively high concentrations of dThd, the authors ascribed the inhibition of FdUrd uptake by dThd to competition for thymidine kinase. Consideration of the contribution of feedback inhibition to the regulation of FdUrd in the presence of other nucleosides has not been adequately addressed. The effects of dThd and IdUrd on FdUrd phosphorylation are important because of the increased use of arterial infusions of FdUrd (48), the presence of significant levels (≤0.8 μM) of dThd in the plasma (37), and the possible use of FdUrd and IdUrd in combination (15–18).

A previous report (49) examined the role of dTTP on FdUrd uptake by using S49 mutants with genetically altered intracellular dTTP pools. The authors found that FdUrd toxicity and its rate of uptake are inversely related to intracellular dTTP pool sizes. One limitation of this approach is the establishment of a direct relationship between total intracellular dTTP pools and enzyme activity. The mutant cells could have other differences such as compartmentalization of dTTP pools (43). In the present study we have circumvented this problem by using 5'-AdThd as a diagnostic tool. As shown before (Fig. 2 and references), the interaction of 5'-AdThd with thymidine kinase is critically dependent on dTTP: 5'-AdThd stimulates enzyme activity in the presence of significant feedback inhibition but acts as a competitive inhibitor in the absence of inhibition by dTTP. Using this approach we have demonstrated that dThd and IdUrd modulate FdUrd activation by substrate competition and, to a significant extent, by feedback inhibition.

It has been shown previously that the salvage of dThd can reduce the cytotoxicity of FdUrd by overcoming the metabolic blockade induced by FdUMP on thymidylate synthetase (50–52). However, activation of FdUrd by thymidine kinase could also be a critical step. In this study inhibition of cell growth by FdUrd correlated with the amount of FdUrd uptake. The fact that the reduction of FdUrd cytotoxicity paralleled the inhibition of FdUrd uptake by dThd, IdUrd, and 5'-AdThd implies that, in addition to replenishment of dTTP pools, the activation step is very important in the modulation of FdUrd cytotoxicity. The results from our study suggest that the antagonistic effects of IdUrd on FdUrd uptake might diminish the expected cytotoxic effects of this combination.

In a previous study, FdUrd and IdUrd resulted in a synergistic cytotoxic combination in T24 cells, a human bladder cancer cell line (18). In the same report, Benson et al. found that 5-fluorouracil potentiated IdUrd cytotoxicity. In light of the present findings, the use of 5-fluorouracil in combination with IdUrd may have advantages over FdUrd since 5-fluorouracil does not depend on activation by thymidine kinase.

The importance of the regulation of thymidine kinase by feedback inhibition can also be illustrated in the case of ribavirin and azidothymidine. Vogt et al. (53) found that ribavirin antagonizes the antiviral effects of azidothymidine in human immunodeficiency virus-infected cells. They suggested that this interaction is probably due to the ribavirin-induced increase in dTTP pools, thus inhibiting the activation of azidothymidine by thymidine kinase. In our study, dThd and IdUrd increased the feedback inhibitor pools and, thus, reduced the activation of FdUrd. In general, in drug combinations in which the agents share a common activating enzyme, antagonism is likely. Furthermore, if the activating enzymes are regulated, then perturbations of the effector pool sizes could have important consequences.

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