A Novel Mechanism of Synergistic Cytotoxicity with 5-Fluorocytosine and Ganciclovir in Double Suicide Gene Therapy

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Abstract
The combination of cytosine deaminase (CD) and herpes simplex virus thymidine kinase (HSV-TK) suicide gene protocols has resulted in enhanced antitumor activity in cultured tumor cells and animal models. In this study, we show that concurrent addition of prodrugs 5-fluorocytosine (5-FC) and ganciclovir (GCV) was less efficacious than sequential treatment in human DU145 prostate carcinoma cells infected with an adenovirus containing a CD/HSV-TK fusion gene. If cells were incubated for 24 hours with 5-FC followed by a 24-hour GCV treatment, GCV triphosphate levels were 2-fold higher, incorporation of GCV monophosphate into DNA was 2.5-fold higher, and growth inhibition was increased 4-fold compared with simultaneous treatment. As expected, cellular dTTP levels were reduced during the 5-FC preincubation. However, dGTP pools also declined parallel to the dTTP decrease. Similar results were obtained when 5-fluorouracil or 5-fluoro-2'-deoxyuridine was used instead of CD/5-FC. These data allowed us to propose a novel hypothesis for the synergistic interaction between CD/5-FC and HSV-TK/GCV treatments. We suggest that the CD/5-FC-mediated reduction of dGTP results in a concurrent decrease of dGTP due to allosteric regulation of ribonucleotide reductase. Because dGTP is the endogenous competitor of GCV triphosphate, depleted dGTP at the time of GCV addition results in increased GCV in DNA and cell kill. In fact, addition of deoxyguanosine during the 5-FC incubation reverses the dGTP depletion, reduces the amount of GCV monophosphate incorporated into DNA, and prevents the CD/5-FC-mediated enhancement of HSV-TK/GCV cytotoxicity. Understanding this mechanistic interaction may help recognize better strategies for creating more efficacious clinical protocols. (Cancer Res 2006; 66(6): 3230-7)

Introduction
Suicide gene therapy is an experimental approach designed to improve the selectivity of cancer treatment (1). The cDNA for a viral or bacterial enzyme (suicide gene) is introduced into tumor cells, enabling them to activate a normally innocuous compound (prodrug) to a cytotoxic form that causes tumor cell death. The most studied of these strategies involves transfer of the bacterial cytosine deaminase (CD) gene or the herpes simplex virus thymidine kinase (HSV-TK) gene into tumor cells, which then selectively sensitize them to 5-fluorocytosine (5-FC) or ganciclovir (GCV), respectively (2, 3). Tumor cells which express CD can selectively convert 5-FC to the anticancer drug 5-fluorouracil (5-FU), which is metabolized by cellular enzymes to 5-FdUMP, resulting in cell death due to inhibition of thymidylate synthase, depletion of dTTP pools, and DNA double-strand breaks (4). Tumor cells expressing the viral HSV-TK are able to initially phosphorylate GCV, leading to an accumulation of its cytotoxic metabolite, GCV triphosphate (GCVTP), and subsequent incorporation into DNA and apoptosis (5–7). Both of these therapies are made more effective by a phenomenon termed the “bystander effect,” which is the ability of suicide enzyme-expressing cells to sensitize neighboring nonexpressing bystander cells to the prodrug. Once produced in CD-expressing tumor cells, 5-FU can diffuse freely across the plasma membrane; however, phosphorylated GCV is highly charged and requires gap junctional channels or other mechanisms of transfer (8–10). The potent cytotoxicity and bystander killing have resulted in excellent activity in many different types of tumor cells in vitro and produced marked tumor regression in several animal models. Whereas clinical trials have shown that these suicide gene vectors can be administered safely, there has been limited efficacy with this approach (1, 11–16). To date, the principal hurdle in cancer gene therapy is the inability to express the suicide gene in a sufficient number of tumor cells.

Because most cancers are typically treated with a combination of drugs and therapeutic modalities, it was realized that the success of suicide gene therapy will likely depend on improved methodolo-

gies or adjuvant treatments. A recent clinical protocol involving multiple therapeutic strategies has been developed for the treatment of men with local recurrence of prostate cancer (15). This experimental protocol employs a novel three-pronged strategy for treating prostate cancer, which includes (a) a lytic, replication-competent adenovirus vector, (b) double suicide gene therapy, and (c) external beam radiation therapy. This sequence has the advantage of enhancing subsequent therapies and has resulted in significantly greater cytotoxicity and tumor growth delay than any of the modalities used individually (17). In this protocol, suicide gene therapy is an adjuvant to radiation therapy and has produced encouraging results (18).

Whereas many laboratories are currently investigating strategies for improved viral vectors and vector delivery, our laboratory has focused on better understanding and improving GCV-mediated cytotoxicity and bystander killing (19–22). Even with the most advanced gene transfer methodologies, the overwhelming majority of the tumor will still consist of nontransduced bystander cells. Optimizing cytotoxicity in this population could be critical to the overall efficacy of this protocol. Several studies, including the above clinical protocol, have integrated the CD/5-FC and HSV-TK/GCV strategies that, when combined together, have been more effective.
compared with the use of either strategy alone (17, 23–25). However, relatively little has been reported about the basis for this interaction. Therefore, we initiated in vitro experiments to elucidate mechanisms for the synergy observed between CD/5-FC and HSV-TK/GCV therapies that we believe would aid in formulating new strategies for improving the clinical application of current double suicide gene therapy protocols.

The data from this study allow us to propose a novel hypothesis for the synergistic interaction between CD/5-FC and HSV-TK/GCV treatments. We suggest that a CD/5-FC-mediated reduction of dTTP levels results in a concurrent decrease of dGTP due to allosteric regulation of ribonucleotide reductase (26). Because dGTP is the endogenous competitor of GCVTP, a depleted dGTP pool at the time of GCV addition can result in increased GCV incorporation into DNA and cell kill (20). In fact, including 2'-deoxyguanosine during the 5-FC treatment reverses the depletion of endogenous dGTP, reduces the amount of GCV incorporated into DNA, and negates the increased GCV-mediated cytotoxicity associated with CD/5-FC.

Materials and Methods

Cell culture and adenoviral transduction. The human DU145 prostate carcinoma cell line was cultured in DMEM medium supplemented with 2 mmol/L L-glutamine and 10% dialyzed fetal bovine serum (Life Technologies, Inc., Grand Island, NY). Cells were maintained in exponential growth in a humidified incubator at 37°C in an atmosphere of 5% CO2 and 95% air. A DU145 clonal cell line was developed from parental cells using a retrovirus vector containing the cDNA for HSV-TK (19).

Construction of the Onyx derived adenovirus vector containing a CD/HSV-TK fusion gene (Ad5-FGNR) has previously been described (17). DU145 cells were plated at a density of 0.5 × 106 per 75-cm2 flask and allowed to grow for ~48 hours. Culture medium was removed and Ad5-FGNR was added at a multiplicity of infection (MOI) between 100 and 500 in 5 mL of serum-free DMEM medium. The efficacy of transduction was assessed by immunocytochemistry using an antibody for CD (27). In these studies, a MOI of 500 resulted in ~85% transduction (data not shown). Following a 2-hour incubation, adenovirus-containing medium was removed and DU145 cells were allowed to grow in complete medium for an additional 24 hours before drug treatment.

Analyses of cellular deoxynucleotide triphosphates and GCV metabolism. Following infection with Ad5-FGNR, DU145 cells were incubated with 1 μmol/L GCV either in combination with or after a 24-hour exposure to 200 μg/mL 5-FC. In some experiments, 100 to 300 μg/mL 2'-deoxyguanosine (Sigma Chemical Co., St. Louis, MO) was included during the preincubation period. Cells were harvested by trypsinization and nucleotides were extracted with ice-cold 0.4 N perchloric acid and neutralized. Endogenous deoxyribonucleotides were initially separated from ribonucleotides on a boronate affinity column (28). Nucleoside triphosphates were then separated and quantitated by strong anion exchange high-performance liquid chromatography using a Waters (Milford, MA) gradient system controlled by Millennium 2010 software as previously described (19). To measure the accumulation of GCVTP, cellular extracts from Ad5-FGNR-infected DU145 cells treated for 0 to 24 hours with 1 mol/L GCV containing ~10% [3H]GCV (Moravek Biochemicals, Inc., Brea, CA), were separated by anion exchange high-performance liquid chromatography. Fractions were collected and radiolabeled GCV nucleotides were quantitated by liquid scintillation spectrometry. To measure the amount of GCV incorporated into DNA, the acid-insoluble cell pellets were washed with 0.4 N perchloric acid and solubilized overnight in 1 N KOH. Incorporation of [3H]GCV into DNA was then estimated by quantitating the amount of radiolabeled GCV by liquid scintillation spectrometry. Data were collected from at least two separate experiments done in duplicate and an unpaired t test was used to determine statistical significance (GraphPad InStat, GraphPad Software, Inc., San Diego, CA) with significance level set at P < 0.05.

Clonogenic survival assays. The sensitivity of DU145 cells stably expressing HSV-TK was measured following a 24-hour exposure to GCV in the presence of 5-FU or 5-fluoro-2'-deoxyuridine (FdUrd). Exponentially growing cells were treated for 24 hours with 0.01 to 10 μmol/L GCV (Cytovene, Syntax, Palo Alto, CA) alone or with 30 μmol/L 5-FU (Sigma Chemical) or 10 μmol/L FdUrd (Sigma Chemical). In some experiments, cell cultures were incubated for 24 hours with either 50 μmol/L 5-FU or 10 μmol/L FdUrd before GCV treatment. In these experiments, drug-containing medium was removed after 24 hours and replaced with fresh growth medium containing GCV. After drug exposure, the cell cultures were trypsinized, counted with a Coulter electronic particle counter, and diluted to ~100 viable cells per 35-mm diameter well in six-well culture dishes. After 10 to 14 days, the resulting cell colonies were fixed in methanol/glacial acetic acid (3:1, v/v), stained with 0.4% crystal violet (Fisher Scientific, Hampton, NH), and visually counted. Cell survival was expressed as a fraction of plating efficiency for untreated cells.

Results

Effect of 5-FC on GCV metabolism. As an initial attempt to understand the mechanism for the synergy observed between 5-FC and GCV, we evaluated the accumulation of GCVTP and the subsequent incorporation of GCVMP into DNA. Human DU145 prostate carcinoma cells were infected with an adenovirus containing a CD/HSV-TK fusion gene (Ad5-FGNR) and treated simultaneously with 1 μmol/L [3H]GCV and 200 μg/mL 5-FC for 24 hours. As illustrated in Fig. 1, the amount of GCVTP, GCVMP in DNA or growth inhibition in cell cultures incubated simultaneously with 5-FC and GCV was not significantly different from that...

![Figure 1. Accumulation of [3H]GCVTP (left), incorporation of [3H]GCVMP into DNA (middle), and growth inhibition (right) were determined in Ad5-FGNR-infected (MOI 500) DU145 cells treated with 5-FC and GCV. Results were calculated following a 24-hour incubation with 1 μmol/L [3H]GCV either alone, together with 200 μg/mL 5-FC, or subsequent to 4 or 24 hours of preincubation with 200 μg/mL 5-FC. Columns, mean; bars, SE. * P < 0.05, GCV treatment versus GCV with a 24-hour 5-FC pretreatment.](image)
with GCV treatment alone. The level of radiolabeled GCVTP was 188 ± 30 in cells treated with both drugs compared with 143 ± 20 pmol/10⁶ cells with GCV alone, and less GCVMP was detected in DNA of cells treated with the combination of drugs (8.4 versus 11.4 pmol/10⁶ cells). Because increases in GCV metabolism were not observed at 24 hours, we evaluated the effect of sequential drug addition. When Ad5-FGNR-infected cultures were treated for 24 hours with 200 μg/mL 5-FC followed by 1 μmol/L [3H]GCV for 24 hours, GCVTP accumulation was 2.6-fold higher compared with GCV treatment alone and incorporation into DNA was also elevated >2-fold (Fig. 1). In addition, exposure to 5-FC for 24 hours before GCV addition resulted in a substantial increase in growth inhibition in contrast to concurrent addition. When administered alone, 5-FC (78 ± 4%) and GCV (72 ± 7%) decreased growth similarly. Compared with GCV treatment, both drugs given simultaneously decreased cell number by 15% whereas sequential addition of 5-FC followed by GCV resulted in a 60% decrease. However, enhanced GCV metabolism and growth inhibition were not achieved with a shorter 4-hour exposure to 5-FC before GCV (Fig. 1). If the length of simultaneous drug exposure was extended to 48 hours, the levels of GCVTP accumulation and incorporation into DNA were similar to the 24-hour sequential treatment (data not shown).

Analysis of cellular deoxynucleotide triphosphate levels. Because 5-FC targets thymidylate synthase and subsequently affects dTTP pools, we measured the effect of 1 μmol/L GCV and 200 μg/mL 5-FC on endogenous nucleotide concentrations and GCVTP over a 24-hour period in DU145 cultures infected with Ad5-FGNR. Consistent with the data in Fig. 1, sequential addition of 3-FC enhanced the activation of GCV, resulting in 2.7-fold more GCVTP accumulation compared with concurrent addition (Fig. 2A and B). Concurrent addition of drugs resulted in little change in dTTP levels over time (Fig. 2A). The dATP pool was elevated 1.8-fold at 24 hours. Although a 35% decrease was observed initially in dGTP pools, values remained unchanged over the rest of the experiment. In Fig. 2B, deoxynucleotide triphosphates (dNTP) were measured following GCV addition, which was preceded by a 24-hour 5-FC incubation. The levels of dTTP, dATP, and dGTP all increased during the course of this experiment and, at 24 hours, were elevated 5.1-, 2.6-, and 3.2-fold, respectively. However, of particular note, at the time of GCV addition, the levels of both dTTP and dGTP were below initial levels observed in Fig. 2A. The effect of the 5-FC incubation is illustrated in Fig. 2C. As expected, addition of 200 μg/mL 5-FC alone decreased the levels of endogenous dTTP. However, this decrease was accompanied by a
corresponding decrease in dGTP concentrations. After a 24-hour 5-FC exposure, dTTP and dGTP pools were reduced by 84% and 90%, respectively.

**Coinculation with 5-FU or FdUrd.** To evaluate whether these effects would be observed with inhibition of thymidylate synthase in the absence of CD gene therapy, we did similar experiments using thymidylate synthase inhibitors (5-FU and FdUrd) in DU145 engineered to express HSV-TK. The concentrations of 5-FU (50 μmol/L) or FdUrd (10 μmol/L) used in the following experiments reduced cell survival by ~50%. A 24-hour incubation with 50 μmol/L 5-FU reduced both dTTP and dGTP by 68% (Fig. 3A) whereas 10 μmol/L FdUrd decreased dTTP and dGTP by 65% and 63%, respectively (Fig. 3B). Overall, the combination of GCV with concurrent and sequential addition of thymidylate synthase inhibitors yielded similar results as CD/5-FC.

A 24-hour incubation with 50 μmol/L 5-FU followed by a 24-hour exposure to 1 μmol/L [3H]GCV produced a 3-fold increase in GCVTP, a 50% increase in GCVMP in DNA, and a 65% decrease in cell growth compared with GCV treatment alone (Fig. 3A). Similar changes were observed in Fig. 3B with 10 μmol/L FdUrd pretreatment; however, GCVTP levels were only elevated ~2-fold. The simultaneous exposure to GCV and either 5-FU (Fig. 3A) or FdUrd (Fig. 3B) resulted in a slight increase in GCVTP (P < 0.05) and a significant reduction of GCVMP incorporation into DNA (P < 0.01) with little effect on cell growth (<10%).

Because sequential addition of these thymidylate synthase inhibitors enhanced the incorporation of GCV into DNA, we wished to determine if this increase would result in improved GCV-mediated cytotoxicity. DU145 cells which stably express HSV-TK were incubated for 24 hours with GCV alone, GCV + 5-FU or FdUrd, or GCV following a 24-hour incubation with 5-FU or FdUrd. Dose-response curves from clonogenic survival assays are shown in Fig. 4. Compared with GCV alone, both simultaneous and sequential drug treatments were significantly different (P < 0.05) at all GCV concentrations with the exception of FdUrd and 10 μmol/L GCV with P = 0.37 and 0.23, respectively. With either thymidylate synthase inhibitor, sequential addition was more cytotoxic than concurrent addition. The IC90 of GCV was decreased from ~0.4 μmol/L to 0.2 μmol/L with concurrent addition and to 0.02 μmol/L with sequential addition of 5-FU (Fig. 4A). The IC90 of GCV was reduced from 0.8 μmol/L to 0.2 and 0.04 μmol/L with concurrent and sequential addition of FdUrd, respectively (Fig. 4B). At 1 μmol/L GCV (the concentration used in Fig. 3A and B), the level of enhanced cytotoxicity with concurrent drug addition was 1.2-fold (5-FU) and 5.4-fold (FdUrd) greater than expected if the drugs were additive. However, with sequential addition, this enhancement is further increased 24- and 35-fold with 5-FU and FdUrd, respectively.

**Effect of 2′-deoxyguanosine on 5-FC and GCV treatment.** The above data show that the sequence of 5-FC (5-FU/FdUrd) preincubation followed by GCV significantly increases the sensitivity of DU145 cells to GCV treatment. Preincubation with 5-FC not only depleted cellular dTTP levels but also equally decreased the level of dGTP. Because dGTP is the endogenous competitor of GCV, a depleted dGTP pool at the time of GCV addition should result in increased GCV incorporation into DNA and cell kill (20). To determine if the observed increase in sensitivity to GCV is due to depletion of dGTP, we included increasing amounts of 2′-deoxyguanosine in similar experiments during the 5-FC preincubation period. As illustrated in Fig. 5, the addition of 100 to 300 μg/mL 2′-deoxyguanosine partially reversed the depletion of dGTP and dTTP caused by 5-FC treatment. In the presence of the added 2′-deoxyguanosine, the 5-FC-mediated increase in dATP was further elevated by up to 2-fold (data not shown). The effects of 2′-deoxyguanosine on GCV metabolism are shown in Fig. 6. Increasing amounts of 2′-deoxyguanosine did not significantly (P > 0.3) alter the elevated GCVTP associated with 5-FC pretreatment. However, the level of GCV incorporation into DNA was significantly reduced (P < 0.003) with the addition of increasing 2′-deoxyguanosine concentrations compared with the ~2-fold increase in the amount of GCVMP in DNA observed.
following 5-FC incubation. If 100 to 300 μg/mL thymidine was included in place of 2’-deoxyguanosine during the 5-FC incubation, similar results were observed (data not shown). Levels of dTTP and dGTP were elevated, GCVMV in DNA was decreased, and GCVTP was unaffected. However, when the same concentrations of thymidine were added with GCV after the 5-FC incubation, GCV phosphorylation and incorporation into DNA were completely eliminated (data not shown). GCV-mediated cytotoxicity was also correspondingly affected by 2’-deoxyguanosine (Fig. 7). Combining 2’-deoxyguanosine with 5-FC increased the survival of DU145 cells following a 24-hour exposure to GCV and negated a majority of the additional cell kill resulting from sequential 5-FC treatment.

Discussion

The excellent antitumor activity observed with HSV-TK/GCV in animal models prompted clinical trials with this strategy (29–32). Whereas this form of suicide gene therapy was safe when administered to patients, lack of efficacy has encouraged combi-

nations with other therapies (1, 11–16). HSV-TK/GCV was combined with another suicide gene therapy, CD/5-FC, because the latter approach also produced strong antitumor activity in animal models (33–35). Several groups have now shown that double suicide gene therapy HSV-TK/GCV and CD/5-FC produces synergistic cytotoxicity in vitro and excellent antitumor activity in animal models including tumor regressions (17, 23–25). However, in some cases, this double suicide gene therapy approach produced only additive (36, 37) or even antagonistic antitumor activity (38). To better understand and clinically use HSV-TK/GCV and CD/5-FC, we have evaluated the mechanistic interaction between these two enzyme/prodrug approaches. The results show that CD/5-FC–mediated alteration of dNTP pools can modulate the incorporation of GCVMV into DNA to increase cytotoxicity, and this is dependent on sequence and duration of drug addition.

The sequential addition of either 5-FU orFdUrd followed by GCV treatment enhanced cytotoxicity above an additive effect by 24- to 35-fold compared with only a 1- to 5-fold increase with simultaneous addition. The enhanced cytotoxicity with concurrent drug addition is similar to the 2- to 3-fold increase observed previously in gliosarcoma cells (25). The results presented here showed that superiority of the sequential addition of 5-FC (24 hours) followed by GCV was due to enhanced incorporation of GCVMV in DNA resulting in higher cytotoxicity. The incubation with 5-FC produced substantial decreases in both dTTP and dGTP pools, and subsequent addition of GCV resulted in an increase in GCVMV accumulation compared with that observed with simultaneous drug addition. Any of these three effects could result in enhanced GCVMV incorporation into DNA. It has been suggested by others that reduced dTTP levels relieves feedback inhibition of mammalian thymidine kinase, which increases salvage synthesis of dTMP from intracellular thymidine (23). Reduction in intracellular thymidine concentrations would allow GCV to compete better in binding to the active site of HSV-TK. To support this hypothesis, the authors included thymidine in their cell extracts and were able to eliminate the increased GCV phosphorylation associated with 5-FC treatment. When thymidine was added to our cell cultures at the same time as GCV (following preincubation with 5-FC), GCVMV accumulation and GCVMV in DNA were also reduced to nondetectable levels. However, thymidine addition during the 5-FC preincubation elevated both dTTP and dGTP but did not affect GCVMV levels and resulted in decreased incorporation of GCVMV in DNA. Thus, these data show that the enhanced GCVMV accumulation was not due to the CD/5-FC–mediated decrease in dTTP pools.

The majority of our data support the concept that decreased dGTP contributed substantially to the enhanced incorporation of GCVMV into DNA. It is likely that dGTP was decreased due to the CD/5-FC–mediated decrease in dTTP. Because dTTP promotes reduction of GDP through allosteric regulation of ribonucleotide reductase, the lower dTTP levels would result in less GDP reduction to dGDP and, thus, lower dGTP levels (26). The decrease in dGTP pools would lessen competition with GCVMV for DNA polymerases. Indeed, the addition of 2’-deoxyguanosine to the 5-FC incubation restored dGTP almost to control levels, resulting in a reduction in GCVMV in DNA and lessened cytotoxicity. Thus, these data strongly suggest that depletion of dGTP by CD/5-FC is an important component contributing to enhanced GCV cytotoxicity.

A role for decreased dGTP mediating enhancement of GCV effects has previously been reported by us using ribonucleotide reductase
inhibitors to increase bystander cell killing with HSV-TK/GCV (20). This synergistic interaction was not dependent on cell type or on level of gap junctional intercellular communication. The mechanism for synergistic bystander cytotoxicity seems to involve a decrease in endogenous dGTP, an increase in GCVTP/dGTP ratio, and increased GCV incorporation into DNA (20). In nude mice colon carcinoma xenografts, the combination of GCV and hydroxyurea significantly delayed tumor growth, in contrast to single-drug treatments which produced tumor growth similar to untreated tumors (39). More recently, we have reported that another inhibitor of ribonucleotide reductase, gemcitabine [2',2'-dihalo-2'-deoxycytidine (dFdCyd)], enhanced bystander cell killing with GCV both in vitro and in vivo. The effects of dFdCyd on GCV-mediated cytotoxicity also seem to be the result of ribonucleotide reductase inhibition and dGTP depletion because incorporation of dFdCyd nucleotides in DNA was actually decreased with GCV addition (22).

Imbalances in cellular dNTP concentrations caused by 5-FC addition could also potentially increase cytotoxicity by inhibiting repair of DNA lesions or delaying the resynthesis of DNA at damaged sites. Perturbations in dNTP pools have been implicated as a trigger for the activation of cellular endonucleases, producing DNA strand breaks and subsequent programmed cell death (40, 41). The number of damaged sites may be augmented by the presence of increased GCVMP in the DNA observed following preincubation with 5-FC and GCV treatment.

The lack of enhancement of cytotoxicity when 5-FC and GCV were administered simultaneously is likely due to the lack of a substantial decrease in dTTP and dGTP levels. However, a 48-hour incubation with both drugs did enhance incorporation of GCVMP into DNA, suggesting that the lengthier drug exposure was able to deplete...
dNTP pools. A prolonged exposure (5 days) to 5-FC and GCV may also have contributed to the synergy observed with concurrent drug addition in an earlier study with gliosarcoma cells (23). It is interesting to note that, whereas modulation with either 5-FC or ribonucleotide reductase inhibitors enhanced GCV-mediated cytotoxicity, 5-FC required sequential drug addition whereas the ribonucleotide reductase inhibitors could be added simultaneously (20, 23). We attribute this to the slow decrease in dTTP and dGTP pools with CD/5-FC, which was greatly diminished with simultaneous addition of GCV, whereas dGTP was decreased rapidly in the presence of ribonucleotide reductase inhibitors alone or with GCV (20, 39). Further support for this concept is illustrated by the fact that accumulation of GCVTP, its incorporation into DNA, and growth inhibition were not altered following a 4-hour preincubation with 5-FC (Fig. 1) when dTTP and dGTP pools were decreased by only 30% and 23%, respectively (Fig. 2C). The thymidine synthase inhibitors 5-FU and ZD1694 have previously been shown to induce synergistic cytotoxicity with HSV-TK/GCV in human (HT29) and mouse (MC38) colon carcinoma cells (42). In this study, the authors showed a 2-fold elevation of GCV incorporation into DNA with either inhibitor. Although this increase was similar to what we observed, they were able to achieve this enhancement with simultaneous incubation. A possible explanation for this difference may be that 5-FU and ZD1694 were used at very high concentrations (10 times the ED50).

The mechanism by which GCVTP levels were increased following sequential addition of 5-FC and GCV is not clear. It is unlikely to occur by regulation of HSV-TK because this enzyme is significantly less sensitive to feedback inhibition compared with mammalian thymidine kinase (43). We have previously reported that the GCVTP levels in SW620 carcinoma cells were increased with the combination of hydroxyurea and GCV, which also reduced thymidine kinase gene therapy model of cancer treatment. Gene Ther 1997;4:244–51.


References


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