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5-Fluoro-1-(2′-Deoxy-2′-Fluoro-β-D-Ribofuranosyl) Uracil Trapping in Morris Hepatoma Cells Expressing the Herpes Simplex Virus Thymidine Kinase Gene

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The planning and individualization of gene therapy with suicide genes such as herpes simplex virus thymidine kinase (HSV-tk) necessitates the assessment of the enzyme activity expressed in the tumor. This can be done by uptake measurements of specific substrates for HSV-tk. Due to the molecular structure of 5-fluoro-1-(2′-deoxy-fluoro-β-D-ribofuranosyl)uracil (FFUdR), it may be a substrate for both the mammalian thymidine kinase and HSV-tk. **Methods:** Using a HSV-tk-expressing rat hepatoma cell line and a control cell line (bearing the empty vector) the uptake of ^{3}H-FFUdR was determined with increasing incubation periods. Furthermore, measurements with graded mixtures of HSV-tk-expressing cells and control cells were made. To elucidate the mechanism of FFUdR transport into cells, a series of inhibition/competition experiments was performed with challenge inhibitors of the nucleoside and the nucleobase transport systems. **Results:** The uptake studies with tritiated FFUdR revealed a 14- to 19-fold higher accumulation in the HSV-tk-expressing cell line compared to the control cell line. While the ^{3}H-FFUdR uptake was 3- to 4-fold higher than the ^{3}H-ganciclovir uptake in the HSV-tk-expressing cells, it was also higher in control cells (5-fold). Furthermore, FFUdR accumulation was linearly correlated with the amount of HSV-tk-expressing cells. FFUdR uptake and growth inhibition by therapeutic doses of ganciclovir were highly correlated, with $r = 0.96$. Inhibition/competition experiments showed that FFUdR is transported mainly by the equilibrative and the concentrative nucleoside transporter but not by the nucleobase transport systems. **Conclusion:** The FFUdR uptake is an indicator of the HSV-tk activity in tumor cells and can be used as a prognostic marker during gene therapy with HSV-tk. The relative merits of ganciclovir and FFUdR as specific substrates for HSV-tk will need to be further explored in vivo.

Key Words: gene therapy; specific substrate; hepatoma; PET

J Nucl Med 1998; 39:1418–1423

Received Jul. 16, 1997; revision accepted Oct. 23, 1997.

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Human gene therapy is a new therapeutic modality that is rapidly progressing into diverse areas of clinical research. One promising gene therapy approach is based on the insertion of a toxin or suicide gene in tumor cells, leading to immediate cell death (toxin gene) or to sensitivity to specific prodrugs (prodrug activation enzyme). The herpes simplex virus thymidine (TdR) kinase (HSV-tk) catalyzes the phosphorylation of nucleoside analogs such as ganciclovir (GCV) and acyclovir, which are poor substrates for the tk of mammalian cells. After incorporation of the phosphorylated metabolites into the DNA, arrest of DNA synthesis and DNA termination occur (*1–4*). Because the in situ transduction efficiency is very low for current viral vectors and the schedule for prodrug administration depends on the knowledge of the suicide enzyme activity induced in the tumor, noninvasive methods for the assessment of this suicide enzyme activity are needed (*5–8*). A selective and potent probe molecule for this purpose would be useful, particularly if labeled with a positron-emitting isotope such as ^{18}F that can be imaged in vivo with PET. Several TdR analogs are selective substrates for HSV-tk and could serve as probes to evaluate the expression of the therapeutic gene. Thymidine analogs phosphorylated by this enzyme would be charged and effectively trapped intracellularly based on a low plasma membrane permeability of the phosphate. With the high specific activity available with PET isotopes (1 mCi/nmol), a tracer-based imaging agent could be administered at nontoxic levels.

To develop a noninvasive method for the evaluation of tumor proliferation, Shields et al. (*9*) studied TdR analogs for their use as a radiotracer for PET examinations. The most promising agent of several TdR nucleosides examined for their ability to image cell proliferation was 5-Fluoro-1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)uracil (FFUdR) (*10*). This molecule displayed high retention in proliferating tissues and in vivo stability and resistance to catabolism, and either of its fluorine substituents might be amenable to labeling with ^{18}F, a convenient isotope for PET imaging. Due to its molecular structure, FFUdR may be a substrate not only of mammalian tk, but also of HSV-tk. Therefore, the compound may be used to assess the HSV-tk activity in an infected tumor.

In this study, FFUdR uptake was measured in a rat Morris hepatoma cell line expressing the HSV-tk gene compared with that in a control cell line that was transfected with an empty retroviral vector. We performed uptake measurements with FFUdR and GCV, an antiviral agent that has been used in clinical gene therapy protocols for treatment of tumors after infection with vectors bearing the HSV-tk gene. We also performed experiments on the metabolism and transport of FFUdR.

MATERIALS AND METHODS

Cells and Growth Conditions

Two Morris hepatoma cell lines were used for all experiments: MH3924LXSNtk8, which shows HSV-tk expression, and MH3924LXSN6, a cell line transfected with an empty retroviral vector (*8*). The cells were cultured in RPMI 1640 medium (Life Technologies, Eggenstein, Germany) supplemented with 20% fetal calf serum, 292 mg glutamine, 100,000 IU penicillin and 100 mg/liter streptomycin in plastic tissue culture flasks (Falcon–Becton Dickinson, Rutherford, NJ) and grown at 37°C, in an atmosphere of 95% air and 5% CO_2. All experiments were performed while the cells were in the logarithmic growth phase. All assays were performed in triplicate.

Uptake Experiment and High-Performance Liquid Chromatography Analysis of Lysates

Three days after seeding six-well plates (Greiner, Solingen, Germany) with 4×10^4 cells, the medium was replaced by 2 ml fresh medium containing 150 kBq ^3H-FFUdR/ml [specific activity = 114 GBq/mmol (3 Ci/mmol); radioactive concentration = 393 μCi/ml; radiochemical purity = >95%]. ^3H-labeled FFUdR was produced as previously described (*10*). After incubation for 1, 2, 4 and 24 hr, the cells were washed twice with ice-cold phosphate-buffered saline, lysed with 400 μl ice-cold 0.6 *M* perchloric acid and removed with a cell scraper. The lysate was centrifuged at 1500 × *g* for 5 min at room temperature. The pellet was washed with 300 μl ice-cold 0.6 *M* perchloric acid, centrifuged at 1500 × *g* for 5 min and resuspended in 500 μl 0.3 *M* NaOH. Subsequently, both supernatants (the acid-insoluble fraction and the acid-soluble fraction) were counted using Pico-Fluor-15 (Canberra Packard, Meriden, CT) and a LSC TRICARB 2500TR (Canberra Packard) scintillation counter. The measured radioactivity was standardized to the viable cell number as determined using a Coulter counter interfaced to a Coulter Channelyzer (Coulter Electronics, Dunstable, United Kingdom).

An uptake experiment was also performed after a 24-hr incubation with 150 kBq/ml ^3H-GCV (Moravek Biochemicals, Brea, CA; specific activity = 662.3 GBq/mmol; radioactive concentration = 37 MBq/ml; radiochemical purity = 99.5%) using identical experimental conditions.

Furthermore, a high-performance liquid chromatography (HPLC) analysis of the acid-soluble fraction was performed. Samples were analyzed by HPLC using a Eurospher column (C_{18}, 5-μm particles, 250 × 4.6 mm inside diameter; Knauer, Berlin, Germany) eluted at 1 ml/min with 2 m*M* NaH_2PO_4 buffer (adjusted to a pH of 3.6 with phosphoric acid). Postcolumn radioactive detection was performed, and radioactivity was detected with a 500-μl liquid scintillation flow-through cell (Canberra A250).

Bystander Experiments

Cells (4×10^4) were seeded in six-well plates as a mixture of control cells (MH3924LXSN6) and HSV-tk-expressing (MH3924LXSNtk8) cells with varying amounts of HSV-tk-expressing cells: 100%, 80%, 40%, 20%, 5% and 0%. Three days later, the cells were incubated for 4 and 24 hr in the presence of 150 kBq/ml ^3H-FFUdR. Lysis of the cells was performed as described in the uptake experiment.

Growth Inhibition

The different mixtures of HSV-tk-expressing and control cells, as described in the bystander experiment, were seeded in six-well plates. After 3 days, the cells were incubated for 72 and 96 hr in the presence of 5 μ*M* GCV, then the cell number was determined using a Coulter ZM counter.

Thymidine Uptake and 5-Fluoro-1-(2′-Deoxy-Fluoro-β-D-Ribofuranosyl)Uracil Uptake After Ganciclovir Treatment

Trypsinized cells (5×10^4) were seeded into each well of the six-well plates. After 3 days, the cells were treated with 5 and 25 μ*M* GCV. Thymidine and FFUdR uptake experiments were performed after 24-hr incubation in the GCV-containing medium. After removal of the GCV-containing medium, the cells were washed, and fresh medium without GCV was added. Thereafter, the cells were pulsed with 185 kBq (*methyl*)-^3H-TdR (Amersham, Braunschweig, Germany; specific activity = 185 GBq/mmol; radioactive concentration = 37 MBq/ml; radiochemical purity = 97.5%) or 75 kBq/ml ^3H-FFUdR. After removal of the medium, the cells were washed twice with ice-cold phosphate-buffered saline. The cells were lysed with 0.5 *M* perchloric acid and removed using a cell scraper. After 30 min on ice, the lysate was removed, and

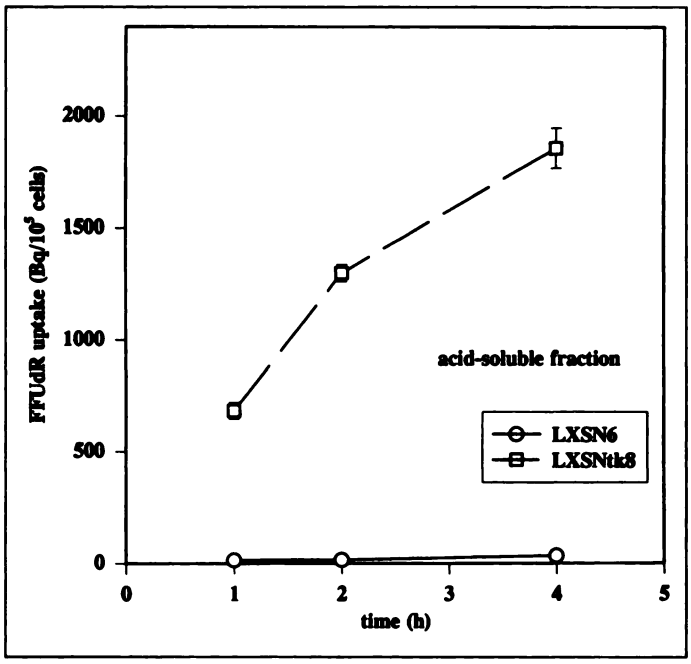

FIGURE 1. 5-Fluoro-1-(2′-deoxy-fluoro-β-D-ribofuranosyl)uracil uptake in acid-soluble fraction after 1-, 2- and 4-hr incubation in control cell line (LXSN6) and HSV-tk-expressing cell line (LXSNtk8). Mean and s.d. are shown (n = 3).

then the pellets were washed after centrifugation with 0.5 *M* perchloric acid and rotated again for 5 min at 0°C. The pellet (acid-insoluble fraction) was resuspended in 1 *M* NaOH at 37°C. Both supernatants (the acid-insoluble fraction and the acid-soluble fraction) were counted as described. The measured radioactivity was standardized to the viable cell number.

Inhibition and Competition Experiment

HSV-tk-expressing cells were trypsinized, and then aliquots of cells (4×10^4) were seeded into six-well plates. Before the uptake experiment, the cells were washed twice with Earle's balanced salt solution (Life Technologies, Inc.) for 30 min, and then the medium was replaced with 1 ml of Earle's balanced salt solution containing 150 kBq/ml ^{3}H-FFUdR. After 10 min of incubation, the cells were washed twice with 1 ml of phosphate-buffered saline on ice and lysed with 500 μl of 0.3 *M* NaOH. For inhibition and competition (*11*), the uptake experiment was performed in the presence of 2.5 μ*M* dipyridamol, 2.5 μ*M* nitrobenzylthioinosine (NBTI), 1 m*M* TdR, 1 m*M* uridine, 1 m*M* chloroadenosine, 1 m*M* uracil and 1 m*M* adenine (all from Sigma, Deisenhofen, Germany). The radioactivity of the lysates was counted as described.

RESULTS

Uptake of 5-Fluoro-1-(2′-Deoxy-Fluoro-β-D-Ribofuranosyl)Uracil and Ganciclovir

After incubating cells for 24 hr, the accumulation of FFUdR in HSV-tk-expressing cells was 14-fold higher in the acid-soluble fraction (representing unbound radioactivity in acid-soluble molecules that are not in DNA and proteins) and 19-fold higher in the acid-insoluble fraction (representing nucleic acids and proteins) than in the control cell line (Fig. 1 and Table 1). The GCV uptake also showed an enhanced ratio of radioactivity in the HSV-tk-expressing cells, by a factor of 21 in the acid-soluble and by a factor of 35 in the acid-insoluble fraction. More than 99% and 91.5% of intracellular FFUdR was found in the acid-soluble fraction after 4- and 24-hr incubation, respectively. In the GCV experiment, 94% of the labeled GCV was found in the acid-soluble fraction.

TABLE 1
Ganciclovir (GCV) and 5-Fluoro-1-(2′-Deoxy-Fluoro-β-D-Ribofuranosyl)Uracil (FFUdR) Uptake in Acid-Soluble and Acid-Insoluble Fraction After 24-Hr Incubation in a Control Cell Line (LXSN6) and a Herpes Simplex Virus Thymidine Kinase-Expressing Cell Line (LXSNtk8)

	Acid-soluble	Acid-insoluble
GCV		
LXSN6	32.9 ± 2.5	2.3 ± 0.2
LXSNtk8	703.8 ± 16.3	80.6 ± 2.0
FFUdR		
LXN6	182.4 ± 22.3	12.1 ± 1.5
LXSNtk8	2494.3 ± 158.9	233.3 ± 16.1

Mean and s.d. are shown (n = 3).

The HPLC analysis of the acid-soluble fraction from control cells revealed that a significant portion of the activity was unmetabolized drug (30%; retention time = 8.8 min) accompanied with a labeled metabolite of FFUdR (70%; retention time = 3.8 min). The observed elution times suggest that the polar metabolite is the expected monophosphate of FFUdR. In the HSV-tk-expressing cells, only the polar metabolite was found.

Bystander Experiments

Bystander experiments were performed with 0%, 5%, 20%, 40%, 80% and 100% of HSV-tk-expressing cells supplemented with the corresponding amount of HSV-tk-negative cells. Beyond the 4-hr incubation period, the progressive uptake of FFUdR closely correlated with the percentage of HSV-tk-expressing cells when the acid-soluble (r = 0.998) and acid-insoluble fractions (r = 0.996) were examined (Fig. 2). A comparable correlation was found with a 24-hr incubation time for the acid-soluble (r = 0.974) and the acid-insoluble (r = 0.9934) fractions.

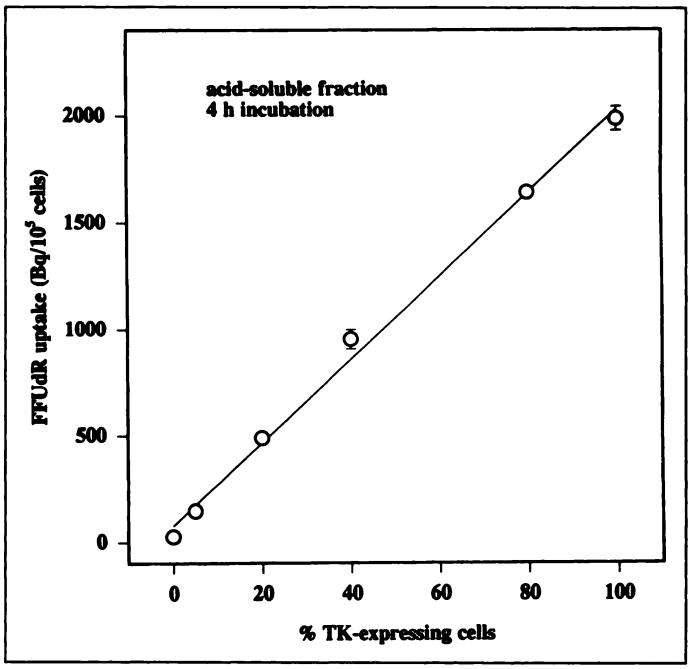

FIGURE 2. 5-Fluoro-1-(2′-deoxy-fluoro-β-D-ribofuranosyl)uracil uptake in acid-soluble fraction in different mixtures of control cells and HSV-tk-expressing cells after 4-hr incubation. Mean and s.d. are shown (n = 3).

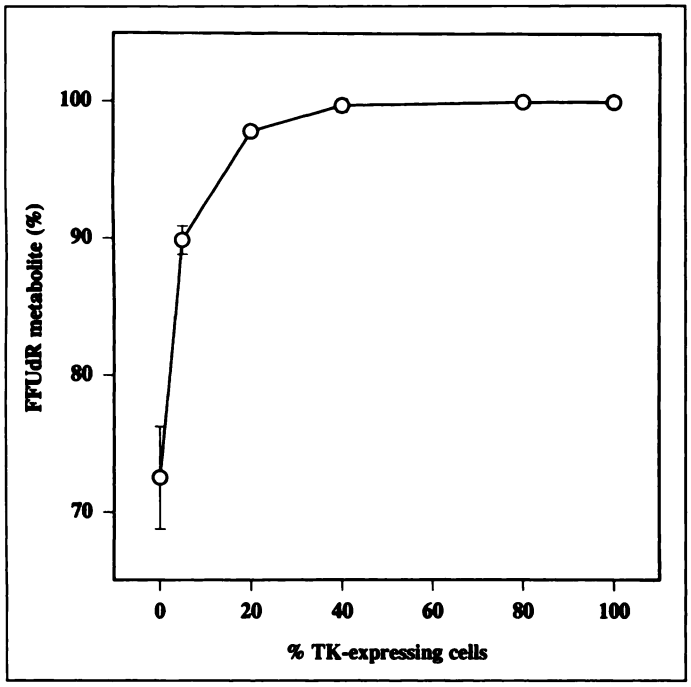

FIGURE 3. High-performance liquid chromatography analysis of acid-soluble fraction in different mixtures of control cells and HSV-tk-expressing cells after 4-hr incubation. Mean and s.d. are shown (n = 3).

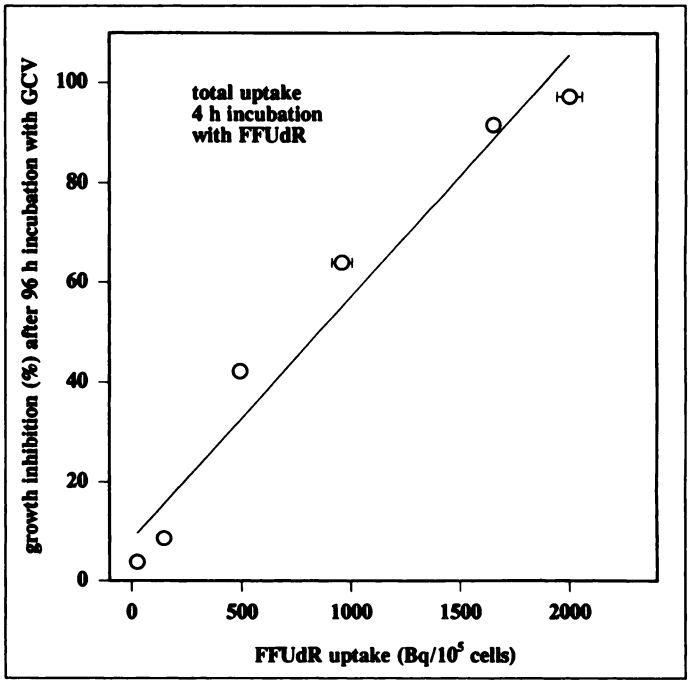

FIGURE 4. Relation of FFUdR uptake after 4-hr incubation and growth inhibition after 96-hr treatment with 5 μM GCV. Mean and s.d. are shown (n = 3).

With increasing percentages of HSV-tk-expressing cells within the mixtures, HPLC analysis of the acid-soluble fractions revealed a linear decrease in the labeled FFUdR content. This was countered by a corresponding buildup of the FFUdR metabolite. In mixtures with more than 20% (24-hr incubation time) or 40% (4-hr incubation time) HSV-tk-expressing cells, only metabolized FFUdR was present (Fig. 3). This is further evidence that the polar metabolite is the expected monophosphate of FFUdR.

After incubation of the above-mentioned mixtures of HSV-tk-expressing cells and control cells with 5 μM GCV, a growth inhibition was observed that was dependent on the amount of HSV-tk-expressing cells. Growth inhibition after 72- and 96-hr exposure to GCV was correlated to FFUdR uptake after 4-hr incubation, with r = 0.946 and r = 0.979, respectively (Fig. 4).

Inhibition/Competition Experiment

To determine the transport characteristics of FFUdR, we studied the uptake of tritiated FFUdR when challenged with known inhibitors/competitors of nucleoside and nucleobases transport systems. Dipyridamole (2.5 μM) caused a 60% decrease in FFUdR uptake within HSV-tk-negative cells (Fig. 5A). Similarly, NBTI accumulation nucleosides, TdR, uridine and chloroadenosine, of the nucleoside transport inhibitors dipyridamol and NBTI (2.5 μM) led to a 47% inhibition. Although chloroadenosine (1 mM) caused an inhibition of 65%, TdR (1 mM) and uridine (1 mM) had no effect.

Relative to FFUdR uptake in HSV-tk-negative cells, we found a 6-fold higher level of accumulation in HSV-tk-positive cells within 10 min of incubation (Fig. 5B). Transmembrane equilibration of FFUdR in these cells was affected by 2.5 μM dipyridamol (83.5%). Incubation with 2.5 μM NBTI resulted in a 48% decrease in FFUdR accumulation. The nucleosides chloroadenosine, TdR and uridine inhibited FFUdR uptake by 93%, 82% and 81%, respectively. As can be seen, there was a sharp contrast in the inhibitions displayed for these nucleosides in HSV-tk-expressing cells relative to those in HSV-tk-negative cells, particularly for TdR and uridine. The three nucleosides are substrates for the sodium-dependent, concentrative transpc carriers. No FFUdR uptake inhibition was seen with the nucleobases adenine (1 mM) and uracil (1 mM).

Thymidine Uptake and 5-Fluoro-1-(2′-Deoxy-Fluoro-β-D-Ribofuranosyl)Uracil Uptake After Ganciclovir Treatment

As anticipated, based on unabated cellular proliferation, labeled TdR uptake in untreated HSV-tk-positive cells was higher than FFUdR uptake, 10-fold in the acid-soluble and 300-fold in the acid-insoluble subcellular fractions (Table 2). In HSV-tk-expressing cells, both TdR and FFUdR uptake showed dose-dependent increases (5 and 25 μM GCV) in radioactivity in the acid-soluble fraction. The corresponding acid-insoluble fractions showed decreased uptake of TdR after GCV treatment. The FFUdR uptake in this fraction was very low and showed a slight increase after GCV treatment, which was not statistically significant as determined with the Mann-Whitney rank sum test (p = 0.1; Table 2).

DISCUSSION

Radiolabeled FFUdR is a promising compound to measure tumor proliferation using PET (*10*). Due to its molecular structure, the substance may be a substrate for HSV-tk and, therefore, may be used for the assessment of suicide enzyme activity. In our study, we examined the accumulation of this TdR analog in a HSV-tk-expressing cell line and a control cell line that was generated by transfection with an empty retroviral vector.

Extraction studies showed a higher total FFUdR uptake in HSV-tk-expressing cells than in the control cell line, with more than 91% of the radioactivity found in the acid-soluble fraction. The 14-fold (acid-soluble fraction) and 19-fold (acid-insoluble fraction) higher uptake in the HSV-tk-positive hepatoma cells indicates that FFUdR is accepted as specific substrate for HSV-tk. This was further indicated by HPLC data, which showed a metabolite that was more polar than FFUdR, which was the only activity to be found in the acid-soluble fraction isolated from HSV-tk-positive cells. Based on the HPLC elution characteristics of the metabolite and the targeted bio-

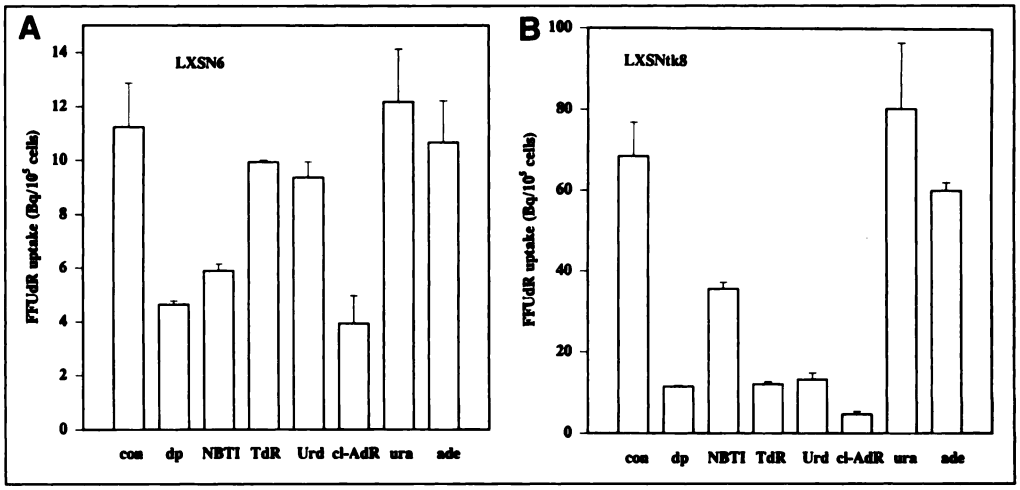

FIGURE 5. 5-Fluoro-1-(2′-deoxy-fluoro-β-D-ribofuranosyl)uracil uptake in control cells (A) and in HSV-tk-expressing cells (B) after 10-min incubation without and in presence of dipyridamole (dp), NBTI, thymidine (TdR), uracil (ura), uridine (urd), adenine (ade) and 2-chloroadenosine (cl-DeR). Mean and s.d. are shown (n = 3).

chemical process for FFUdR, the metabolite is believed to be the 5′-O-monophosphate of FFUdR. The control cells showed 70% of the radioactivity as metabolized FFUdR. This shows that FFUdR is a substrate not only of HSV-tk but also of the host tk. Phosphorylation of FFUdR to its monophosphate by HSV-tk would result in production of an intracellular, negatively charged species that would not be expected to cross the plasma membrane. Therefore, phosphorylation of FFUdR should result in intracellular trapping of FFUdR. Since a prominent time-dependent buildup of activity is found in the acid-soluble fraction derived from HSV-tk-positive cells, it is also reasonable to assume that retrograde dephosphorylation of FFUdR does not occur on a comparable time scale. The amount of intracellular FFUdR can be expected to be a function of the phosphorylating capacity by both the host tk and the HSV-tk. By measuring FFUdR uptake before and after infection with the HSV-tk-bearing virus system, it may be possible to estimate the HSV-tk activity induced in the tumor. Therefore, we compared FFUdR uptake to the accumulation of GCV, which is commonly applied in gene therapy experiments.

In comparison to GCV, FFUdR showed a higher total uptake in HSV-tk-positive cells, as well as in control cells. Therefore, an in vivo PET examination may show more uptake of labeled FFUdR also in unmodified cells. This could result in a higher background activity with respect to the assessment of HSV-tk expression. Therefore, the ratio of FFUdR uptake in HSV-tk-positive cells to that in control cells is of critical importance for PET measurement. Ganciclovir showed a slightly better uptake ratio in HSV-tk-expressing cells versus control cells. However, in our study we used ^{3}H-labeled GCV, and it is not clear whether labeling with ^{18}F, as described by Monclus et al. (*12*), will change the accumulation of the compound in vitro. Furthermore, an in vivo comparison of both tracers is needed. Ganciclovir, as well as FFUdR, undergoes little degradation and is excreted mainly unchanged in the urine (*10,13*). Fluorinated GCV can be expected to show similar pharmacokinetics. Therefore, this compound and FFUdR should be comparable, provided that fluorinated GCV shows a similar affinity to HSV-tk as nonfluorinated GCV.

TABLE 2
Thymidine (TdR) and 5-Fluoro-1-(2′-Deoxy-Fluoro-β-D-Ribofuranosyl)Uracil (FFUdR) Uptake in Acid-Soluble and Acid-Insoluble Fraction After 24-Hr Incubation of the Herpes Simplex Virus Thymidine Kinase or 25 μM Ganciclovir (GCV)

	Acid-soluble	Acid-insoluble
TdR		
Control	1838.9 ± 113.3	1763.2 ± 239.7
5 μM GCV	3658.7 ± 206.5	647.2 ± 17.4
25 μM GCV	4406.8 ± 379.7	263.9 ± 50.9
FFUdR		
Control	211.8 ± 98.2	4.1 ± 1.4
5 μM GCV	282.4 ± 80.2	8.2 ± 0.8
25 μM GCV	309.1 ± 100.4	8.7 ± 0.4

Mean and s.d. are shown (n = 3).

Due to the low in vivo transduction rate, repeated infection of the tumor with the HSV-tk suicide gene is necessary to reach a therapeutic level of enzyme activity. However, not all tumor cells have to be infected with a recombinant retrovirus containing the HSV-tk gene to achieve tumor regression, a phenomenon that is called the bystander effect. Possible mechanisms are transfer of the phosphorylated GCV metabolite via gap junctions and uptake of the phosphorylated GCV metabolite via apoptotic vesicles (*14,15*). Experiments with mixtures of varying amounts of HSV-tk-expressing cells and control cells were performed to simulate a varying amount of intratumoral HSV-tk activity in vitro. In these experiments, we evaluated the proposal that the total accumulation of radiolabeled FFUdR in the whole tumor is an indicator of HSV-tk activity. The uptake of FFUdR, as well as its metabolic conversion, which is directly related to the percentage of HSV-tk-expressing cells present, indicates a dependence of FFUdR uptake on the intratumoral HSV-tk activity (Figs. 2 and 3). Therefore, FFUdR uptake measured after infection of tumor tissue with a vector bearing the HSV-tk gene is an indicator of HSV-tk expression. Furthermore, the growth inhibition after longer incubation periods (72 and 96 hr) with a therapeutic concentration of GCV and FFUdR uptake after 4-hr incubation were highly correlated. Consequently, measurement of FFUdR may be used to predict the outcome of gene therapy with the HSV-tk/GCV system.

A pharmacokinetic analysis of FFUdR accumulation presupposes basic information of the transport mechanism. Therefore, inhibition/competition experiments were performed with different inhibitors and competitors of nucleoside transporters. In a large number of tissues, nucleosides permeate the plasma membrane by a mechanism of facilitated diffusion (*16–18*) that is energy independent and operates bidirectionally in a nonconcentrative way. Two distinct equilibrate transporters with broad substrate specificity (es and ei) have been described. The es carrier is strongly inhibited by nanomolar concentration of NBTI; the ei carrier has a low sensitivity (micromolar), and both are inhibited by nanomolar concentrations of dipyridamol.

A second basic type of nucleoside transport, a concentrative

sodium- and energy-dependent nucleoside transport system, has been observed in several tissues and cell lines. Three principal Na^+-dependent carriers that are highly resistant to inhibition by NBTI and dipyridamol have been distinguished on the basis of substrate specificity. The first carrier, cif, is purine specific, with guanosine and formycin B as model substrates. The second carrier, cit, transports preferentially various pyrimidine nucleosides (*19*). Uridine and adenosine are transported by both carriers. The third carrier, cib, has broad specificity for both purine and pyrimidine nucleosides.

In both cell lines, we found evidence for equilibrative, facilitated nucleoside transport for FFUdR. The inhibition of FFUdR permeation by chloroadenosine showed additionally a concentrative sodium- and energy-dependent nucleoside transport system. Transport via cit carrier is less prominent because TdR as model substrate showed no significant influence on FFUdR uptake in the control cell line (Fig. 5A). Uridine had a smaller effect on the influx of FFUdR in the control cells (Fig. 5A). Therefore, it is possible that, in the control cell line, FFUdR permeated the cell membrane via the cib carrier because of its broad substrate specificity. However, competition between the unlabeled nucleosides and FFUdR not only for the transport but also for the tk might be involved in the measured effects.

Because the contribution of the nucleobase carriers to GCV transport has been described (*20*), the competition of FFUdR uptake for the nucleobase transport systems was also performed using uracil and adenine. In both cell lines, adenine and uracil showed no influence on FFUdR uptake, excluding a significant permeation of FFUdR via a nucleobase transport system.

To examine whether FFUdR uptake can be used as a measure of therapeutic efficacy, we performed ^{3}H-TdR and ^{3}H-FFUdR uptake experiments after GCV treatment. Incubation with GCV caused a decrease in TdR incorporation into the nucleic acid fraction. This demonstrates that GCV is toxic to the cells bearing the HSV-tk activity and interferes with their proliferation. In contrast, FFUdR uptake is not decreased after GCV therapy. The compound is not incorporated in significant amounts into the nucleic acid fraction. Therefore, FFUdR is not useful for the measurement of DNA chain termination during treatment with HSV-tk and GCV. However, after longer periods of treatment, a decrease in host tk activity may be observed in vivo by a decrease in FFUdR uptake in these tumors as an effect of therapy on tumor proliferation.

CONCLUSION

In our studies, we were able to show that FFUdR is a good substrate for HSV-tk expressed in rat Morris hepatoma cells. We observed high retention of FFUdR in the HSV-tk-expressing tumor cells compared to the control cell line. Because the FFUdR accumulation and the amount of HSV-tk-expressing cells are correlated, the effectiveness of an infection and suicide gene expression can be evaluated using PET and ^{18}F-labeled FFUdR. The correlation of FFUdR uptake and growth inhibition after incubation with GCV shows that the measurement of FFUdR uptake may also be used to attempt a prediction of therapy outcome. FFUdR is not incorporated efficiently into the DNA. Therefore, it cannot be used as a marker of DNA chain termination after GCV treatment. The transport of FFUdR in the tumor cells is done by the equilibrative and also by the concentrative nucleoside transport systems.

ACKNOWLEDGMENTS

This work was supported in part by Grant CA-39566 from the National Cancer Institute.

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