Modulation of IdUrd-DNA Incorporation and Radiosensitization in Human Bladder Carcinoma Cells

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ABSTRACT

5'-Amino-5'-deoxythymidine (5'-AdThd) has been demonstrated previously to antagonize dTTP-mediated feedback inhibition of purified thymidine kinase from 647V, a human bladder cancer cell line. Low concentrations of 5'-AdThd (3-30 μM) have also been shown to stimulate cellular uptake of iododeoxyuridine (IdUrd) in 647V cells at clinically relevant IdUrd concentrations (2 μM). We report that the combination of 30 μM 5'-AdThd plus 2 μM IdUrd results in a significant increase of IdUrd replacement of thymidine (dTd) (18%) in the DNA of 647V cells over that obtained by exposure to 2 μM IdUrd alone (7.9%). However, increasing the 5'-AdThd concentration to 300 μM inhibited the incorporation of IdUrd into DNA (3%). IdUrd-induced radiosensitization of 647V cells, as measured by clonogenic survival, was enhanced by coincubation with 30 μM 5'-AdThd, while 300 μM 5'-AdThd reduced the IdUrd radiosensitization. Additionally, radiation-induced single strand break generation when IdUrd was incorporated into 647V DNA, as measured by rapid alkaline elution, was also enhanced by coincubation with 30 μM 5'-AdThd, while 300 μM 5'-AdThd resulted in a decrease in the number of single strand breaks produced. In T24, another bladder cancer cell line, and SV-HUC-TT1, a tumorigenic cell line derived from SV-HUC, 3-10 μM 5'-AdThd was also able to enhance IdUrd replacement of dTdT in DNA. However, no stimulation of dTdT replacement by 5'-AdThd occurred in SV-HUC, a prototypic "normal" bladder urothelial cell line. Since 5'-AdThd is not a substrate for mammalian thymidine kinase and has little or no cytotoxicity in vitro and in vivo, it may be a selective modulator of IdUrd radiosensitization of human bladder carcinoma and should be tested in vivo.

INTRODUCTION

IdUrd is an analogue of the nucleoside dThd. First synthesized by Prussoff (1) as a cytotoxic antineoplastic agent, IdUrd has been shown to function as a radiosensitizing agent when incorporated into the DNA of mammalian cells (2-4) and as an effective clinical radiosensitizer in selected tumor sites (5, 6). A direct correlation between the increase in level of radiosensitivity (2-3 times) and the level of IdUrd incorporation into the DNA of the cell (up to 30%) has been established in some normal and malignant mammalian cells in vitro (3, 7-9). The in vitro effects of IdUrd including radiosensitization can be abolished by inhibiting its incorporation into DNA (10).

The first step in IdUrd cellular metabolism is its conversion to IdUMP by the nucleoside salvage enzyme, TK. The Km for IdUrd is 2 μM and is the rate-limiting step in IdUrd metabolism (11). IdUMP is then sequentially phosphorylated to IdUTP by thymidylate kinase. Thymidine kinase is regulated by the metabolic end products, dTTP and IdUTP, through feedback inhibition. The regulation of TK by IdUTP and dTTP may restrict the levels of IdUTP which may be incorporated into DNA. 5'-AdThd is also an analogue of dThd. While not a substrate of mammalian TK (12), 5'-AdThd is a competitive inhibitor of TK (13, 14). Recent studies have revealed that 5'-AdThd exhibits a dTTP-dependent interaction with TK (15, 16). In the absence of dTTP, 5'-AdThd functions as a competitive inhibitor of TK in cell-free systems. However, when dTTP is present and the enzyme is inhibited by feedback, 5'-AdThd partially reverses the inhibition, apparently through a preferential binding at the regulatory site of the enzyme (16). 5'-AdThd shows little toxicity in vitro (17) and in vivo (18).

The ability of 5'-AdThd to increase TK activity in the presence of dTTP is apparent in both purified enzyme preparations and in nucleoside uptake studies in intact cells (16). Interestingly, the ability of 5'-AdThd to increase the uptake of dThd and IdUrd significantly in intact cells is dose dependent and also appears limited to the malignant human bladder cell lines (647V and T24) compared to normal bladder urothelial cells (SV-HUC) (11, 19).

In clinical studies of radiosensitization, IdUrd has been administered as both i.v. bolus injections and as continuous i.v. infusions. Steady-state IdUrd plasma concentrations of 1.8-2.0 μM have been obtained using a prolonged continuous i.v. infusion (up to 2 weeks) at the maximum tolerated dose of 1000 mg/m² with moderate myelosuppression and stomatitis reported as systemic side effects (20, 21). IdUrd, when given for 14 days as a continuous infusion at 1000 mg/m²/day, produced substitution of dThd in the DNA of granulocytes of 7-17% (mean, 11%) (8, 22).

The ability of 5'-AdThd to antagonize the dTTP-mediated feedback inhibition of purified thymidine kinase, and the specific stimulation of IdUrd uptake in tumor cells at clinically achievable IdUrd concentrations, led us to study the possibility of using 5'-AdThd to increase the incorporation of IdUrd and the in vitro radiosensitization in 647V, a human bladder carcinoma cell line. Additionally, we studied the effect of 5'-AdThd on the incorporation of IdUrd into DNA in two other human bladder carcinoma cell lines, T24 (23) and SV-HUC-TT1 (24), and an SV40-transformed normal human bladder urothelial cell line, SV-HUC (25).

MATERIALS AND METHODS

Chemicals and Drugs

5'-AdThd was obtained from Sigma Chemical Co. (St. Louis, MO). IdUrd and dThd were purchased from Calbiochem Corp. (La Jolla, CA). [methyl-3H]dThd, [methyl-14C]dThd, and [6-3H]IdUrd were purchased from Moravek Biochemicals, Inc. (Brea, CA). Other chemicals were purchased from Sigma or Aldrich Chemical Co., Inc. (Milwaukee, WI). Enzymes for dThd replacement assays were purchased from Sigma.

Cell Culture

All tissue culture media, supplements, and related chemicals were obtained from GIBCO (Grand Island, NY).
647V, a human bladder cancer cell line, was obtained from Dr. E. C. Borden of this department and maintained in Eagle's MEM supplemented with 10% fetal bovine serum, 1 mM nonessential amino acids, 1 mM glutamine and NaHCO₃ (MEM+). 647V has a 24-h doubling time and a PE of 40–60%.

T24. T24 cells, another human bladder carcinoma cell line with a 19-h doubling time and a PE of 45–50%, were obtained from the American Type Culture Collection (Rockville, MD) (23). The culture was maintained in MEM+.

SV-HUC. The SV-HUC cell line was obtained from Dr. C. A. Reznikoff, of this department, as an immortalized "normal" human bladder cell line (25). SV-HUC has a 40-h doubling time and a PE of 35–45%. The culture medium was changed from supplemented F-12 medium to MEM+ medium supplemented with 5 μg/ml insulin, 1 μg/ml hydrocortisone, 5 μg/ml human transferrin, and 2.7 mg/ml dextrose. This change was made to remove the 2 μM dThd present in F-12 medium. No significant change of either the growth rate or plating efficiency was observed by the change in medium.

SV-HUC-TTI. The SV-HUC-TTI cell line was also obtained from Dr. C. A. Reznikoff (24). SV-HUC-TTI has a 40-h doubling time and a PE of 35–45%. SV-HUC-TTI is a 3-methylcholanthrene-transformed tumorigenic cell line derived from SV-HUC. Like SV-HUC, this cell line was transferred to supplemented MEM+ with no significant change in growth rate or PE.

Cells were incubated in a Forma model 3315 trigas processor incubator (Forma Scientific, Inc., Marietta, OH) in a humidified, 5% CO₂ atmosphere at 37°C. Cells were passaged twice weekly. Mycoplasma contamination was checked regularly using GIBCO MycoTest kit (GIBCO, Grand Island, NY).

**Drug Treatment**

Log phase cells were detached from 75-cm² flasks using 0.1% trypsin in PBS-EDTA and resuspended in fresh medium. Cell number was determined using a Elzone model 180/H particle counter (Particle Data, Inc., Elmhurst, IL). Cells (2 x 10⁵) were inoculated into 25-cm² flasks (Costar, Inc., Cambridge, MA) containing 5 ml of medium minus NaHCO₃ and buffered with 25 mM 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid, pH 7.4. The flasks were sealed and incubated overnight at 37°C. Medium was aspirated and fresh medium containing the appropriate drug combination was added. The flasks were incubated at 37°C with IdUrd ± 5'-AdThd for 30 h (647V and T24) or 48 h (SV-HUC-TTI and SV-HUC). These incubation times were chosen to ensure that the cells were exposed for at least 1 cell doubling. No significant effect on cell growth was found with any of the drug combinations during these incubation times in these 4 cell lines. The cells were trypsinized, resuspended in 2.5 ml of fresh MEM+, and counted. Upon exposure of the cells to IdUrd, all samples were processed under yellow fluorescent light to prevent possible photolysis of the DNA.

**Clonogenic Assay**

Ten serial dilutions of the 647V cell suspensions were made in MEM+. An appropriate number of cells were plated into 60 x 20-mm dishes (Becton Dickinson and Co., Lincoln Park, NJ) containing 4 ml of MEM+. Dishes were immediately irradiated using a J. L. Shepherd and Associates model 109 cesium irradiator (Glendale, CA) at a dose rate of 7.5 Gy/min. The dishes were then incubated for 10–14 days at 37°C in a humidified 5% CO₂ atmosphere. Dishes were stained with 0.5% crystal violet (Sigma) in methanohacetic acid (3:1). Colonies containing >50 cells were scored using a Belco plaque viewer (Belco, Vineland, NJ). Each experimental condition in the 647V cells for clonogenic survival was performed in triplicate. SERS were determined using the radiation dose required to produce 1% survival for each treatment condition.

**Rapid Alkaline Elution**

Cells were prepared as described above except that the 647V cells were incubated with 0.01 μCi/ml of [³H-methyl]dThd, [methyl-¹⁴C]-dThd, or [6-³H]IdUrd. methyl-¹⁴C]dThd was used as an internal standard due to the unavailability of [³C]IdUrd. The method used was a modification of that used by Kohn et al. (27). Rapid alkaline elution (40 min) was used to minimize the formation of alkaline-labile sites in IdUrd-substituted DNA (28). Cells (1 x 10⁶) were resuspended in MEM+ on ice. Irradiated samples received 6 Gy of γ-irradiation. Cells were layered onto 0.2-μm Nuclepore polycarbonate filters (Nuclepore Corp., Pleasanton, CA) and then washed twice with 5 ml of ice-cold PBS. The cells were lysed with 2 x 5-ml aliquots of lysis solution containing 20 mM EDTA-1% sodium dodecyl sulfate, pH 10.0. Samples were incubated for 1 h at room temperature in the presence of 0.5 mg/ml proteinase K and 0.1 mM CaCl₂ in the lysis solution. DNA was eluted at pH 12.2 with 2% (v/v) tetratropylammonium hydroxide containing 0.1% sodium dodecyl sulfate and 20 mM EDTA. Seven 5-ml fractions were collected at a flow rate of 0.7 ml/min. Radioactivity remaining in the filter holder and lines was recovered as a separate fraction. Filters were heated to 65°C in 0.4 ml of 1 N HCl and cooled, and the volume was brought to 5 ml with elution buffer. All samples were mixed with 10 ml of EcoLite (ICN Radiochemicals, Irvine, CA) for scintillation spectrophotometry.

**Thymidine Replacement**

Preparation and analysis of samples for HPLC from the 4 different cell lines were performed using a modification of the method of Belanger et al. (20). Treated cells were harvested by centrifugation at 250 x g for 10 min in a Beckman GPR centrifuge (Beckman Instruments, Inc., Fullerton, CA). Cells were washed twice in cold PBS, and the pellet was resuspended in 0.5 ml of distilled H₂O and transferred to 1.5-ml microcentrifuge tubes. One-half ml of 10% TCA was added to each sample to precipitate the macromolecules. Samples were vortexed and pelleted. The pellets were washed once with 10% TCA, resuspended in 100 μl of 0.5N NaOH, and incubated for 90 min at 37°C to hydrolyze the RNA. The samples were precipitated with 1.0 ml of 10% TCA and pelleted. This was followed by one 10% TCA wash. The pellet was then digested in 400 μl of 25 mM KH₂PO₄, pH 7.45, containing 2 mM MgCl₂, 50 units of DNase, 135 units of alkaline phosphatase, and 700 μg of phosphodiesterase. The mixture was vortexed and incubated overnight at room temperature.

**HPLC**

Samples were filtered through Ultrafree-MC ultrafiltration units (Millipore Corp., Bedford, MA). HPLC analysis was performed using a Waters W600 solvent delivery system with a CI₄µ Bondapak RCM 8 x 10 cartridge system (Waters, Milford, MA). Samples were eluted with a 100 mM sodium acetate, pH 5.45-7% acetonitrile solvent system (20). Peaks were detected using a Waters model 490E programmable multiple wavelength detector set at 288, 267, and 254 nm. Peaks were quantified using a Waters model 745 data module. Standard curves were generated for dThd and IdUrd using authentic samples (Sigma).

**RESULTS**

**Thymidine Replacement.** IdUrd replacement of dThd in DNA of 647V cells after 30 h of drug exposure occurs in a concentration-dependent manner that is linear up to 4 μM IdUrd (Fig. 1). Above 4 μM IdUrd, the response begins to level off, with the maximum dThd replacement occurring at approximately 16 μM IdUrd. The limitation of IdUrd replacement of dThd does not appear to be a result of a significant IdUrd-induced increase in the doubling time of 647V (data not shown).

The effect of 5'-AdThd on dThd replacement by IdUrd in DNA of 647V cells at a clinically achievable steady-state concentration (2 μM) is presented in Figs. 1 and 2. Incubation of 647V cells with 2 μM IdUrd showed a 7.9% replacement of dThd by IdUrd. Coincubation of 647V with 2 μM IdUrd and 30 μM 5'-AdThd increased the IdUrd replacement of dThd to 18%. When the 5'-AdThd concentration was increased to 300 μM, IdUrd replacement of dThd was reduced to 3%. 5'-AdThd alone had no effect on either the doubling time or the plating...
efficiency of 647V cells. This biphasic effect of 5'-AdThd on IdUrd replacement of dThd directly correlates with the effect of 5'-AdThd on TK activity from purified enzyme preparations (15, 16, 19).

Clonogenic Assays. The effect of 2 μM IdUrd ± 30 or 300 μM 5'-AdThd on the radiation survival of 647V is shown in Fig. 3. Radiation survival was decreased with preincubation for approximately 1 doubling time (30 h) with 2 μM IdUrd (SER = 1.46) and to a greater extent with 2 μM IdUrd plus 30 μM 5'-AdThd (SER = 1.68). However, coincubation with 2 μM IdUrd and 300 μM 5'-AdThd resulted in less IdUrd-induced radiosensitivity (SER = 1.22) compared to IdUrd alone. 5'-AdThd (30 or 300 μM) alone had no radiosensitizing effect on 647V cells. SERs were determined at 1% cell survival using survival following radiation alone as the control.

Single Strand Breaks. The effect of IdUrd alone or in combination with 5'-AdThd on radiation-induced SSB generation is shown in a representative rapid alkaline elution profile in Fig. 4. Neither IdUrd, 5'-AdThd, nor the combination of the two drugs resulted in the generation of spontaneous SSBs. IdUrd (2 μM) plus 6 Gy of γ-radiation increased the number of SSBs generated over that of radiation alone (6 Gy). The combination of 2 μM IdUrd plus 30 μM 5'-AdThd resulted in a further increase in the number of radiation-induced SSBs. Increasing the 5'-AdThd concentration to 300 μM reduced radiation-induced SSBs to almost that of the irradiated control, thus eliminating the radiosensitizing effect of IdUrd. Neither 30 μM 5'-AdThd alone (data not shown) nor 300 μM 5'-AdThd alone showed any radiosensitizing effect as measured by rapid alkaline elution.

Thymidine Replacement in Other Bladder Cell Lines. A comparison of the effect of 5'-AdThd on IdUrd replacement of dThd in the DNA of 647V (30 h), T24 (30 h), SV-HUC-TT1 (48 h), and SV-HUC (48 h) cells is presented in Fig. 5 as % control dThd replacement versus 5'-AdThd concentration. In the three tumorigenic cell lines, 647V, T24, and SV-HUC-771,
replacement of approximately 22% following exposure for 1
647V cells is a saturable process (Fig. 1) with a maximal
osensitization in tumor cell lines.

bladder cancers compared to normal urothelium (11, 18), we
questioned whether S'-AdThd would also increase IdUrd radi
alone. Additionally, since previous reports demonstrated that
5'-AdThd may further enhance IdUrd cellular uptake in human
than adjacent normal bladder urothelium based on cell kinetics
bladder tumors should incorporate several-fold more IdUrd
expected from these results that high grade invasive human
creases in dThd replacement of 220, 152, and 139% of control
T24, SV-HUC-TT1, and SV-HUC cells. Exposure times for 647V and T24 were
30 and, for SV-HUC-TT1 and SV-HUC, 48 h.

coincubation with 2 µM IdUrd and 5'-AdThd produced in-
creases in dThd replacement of 220, 152, and 139% of control
at 30, 3, and 3 µM, respectively. No increase in the replacement
dThd was observed in SV-HUC cells as a result of coincu-
biation with 5'-AdThd. Inhibition of dThd replacement by
IdUrd in SV-HUC, T24, and SV-HUC-TT1 was observed as
the 5'-AdThd concentration increased >10-30 µM.

DISCUSSION
The goal of this study was to investigate the possibility of
using 5'-AdThd to increase IdUrd-induced radiosensitivity in
human bladder cancer cell lines. IdUrd has been shown to be
an effective clinical radiosensitizer in selected tumor sites such
as glioblastoma multiforme and high grade soft tissue and bone
sarcomas which are actively proliferating tumors surrounded
by nondividing normal tissues (6, 7, 29, 30). In contrast, pre-
vious studies of locally advanced head and neck cancers using
BrdUrd, a structurally similar radiosensitizing compound,
showed sensitization of both tumor and adjacent normal prolifer-
ating tissue (oropharyngeal mucosa), resulting in no therapeu-
tic gain for radiosensitization (31).

In vitro studies using IdUrd alone have shown a direct cor-
relation between radiosensitivity and the amount of IdUrd
incorporation into the DNA of a cell (3, 7-9). In this in vitro
model, we are determining whether biochemical modulation of
IdUrd metabolism might lead to further radiosensitization. In
human subjects, the normal bladder urothelium is known to be
slowly dividing, whereas bladder tumors, particularly high grade
tumors, similar to 647V, T24, and SV-HUC-TT1, are dividing
more rapidly. Using the BrdUrd monoclonal antibody immu-
noperoxidase methodology, a recent study correlated histolog-
ical grade and the % S phase in human bladder cancer in situ
(32). The average % S phase for noninvasive and invasive
tumors was <10% and >20%, respectively. Thus, it would be
expected from these results that high grade invasive human
bladder tumors should incorporate several-fold more IdUrd
than adjacent normal bladder urothelium based on cell kinetics
alone. Additionally, since previous reports demonstrated that
5'-AdThd may further enhance IdUrd cellular uptake in human
bladder cancers compared to normal urothelium (11, 18), we
questioned whether 5'-AdThd would also increase IdUrd radi-
osensitization in tumor cell lines.

We report that IdUrd replacement of dThd in the DNA of
647V cells is a saturable process (Fig. 1) with a maximal
replacement of approximately 22% following exposure for 1
cell doubling time. 5'-AdThd has been shown to antagonize the
dTTP-mediated feedback inhibition of TK (15). This effect has
been mirrored in a stimulation of the uptake of dThd and IdUrd
in 647V and T24 cells and appears to be specific for malignant
human bladder cell lines (11, 18). Incubation of 647V and T24
cells with the combination of 2 µM IdUrd plus 3-30 µM 5'
AdThd increases the replacement of dThd by IdUrd over that
of IdUrd alone (Fig. 5). The increase in dThd replacement by
the IdUrd plus 5'-AdThd over that produced by 2 µM IdUrd
alone increased the effective IdUrd concentration from 2-14
µM in 647V cells (Fig. 1). The biphasic effect of 5'-AdThd on
TK activity reported by Vazquez-Padua et al. (16) was reflected
in the decreased level of IdUrd replacement of dThd in cells
exposed to 2 µM IdUrd plus 300 µM 5'-AdThd (Figs. 1, 2, and
5).

The correlation between IdUrd incorporation into DNA and
radiation damage is reflected in SSB production as measured
by rapid alkaline elution. An increased number of SSBs were
generated in cells incubated with the combination of 2 µM
IdUrd, 30 µM 5'-AdThd, and γ-irradiation over those produced
by IdUrd plus irradiation in 647V cells (Fig. 4). 5'-AdThd (30
or 300 µM) plus irradiation or any unirradiated combination of
IdUrd plus 5'-AdThd had no effect of the alkaline elution of
647V DNA. Thus, IdUrd-related radiation damage to DNA in
647V cells was increased by coincubation of the cells with 30
µM 5'-AdThd.

The response demonstrated by 647V cells to the combina-
tion of IdUrd with or without 5'-AdThd with respect to dThd
replacement and SSB generation was also found in vitro by
clonogenic survival assays (Fig. 3). Radiosensitization of 647V
cells by 2 µM IdUrd is enhanced by coincubation with 30 µM
5'-AdThd. Increasing the 5'-AdThd concentration to 300 µM
while maintaining an IdUrd concentration of 2 µM resulted in
a decrease in the radiosensitivity exhibited by 647V cells. 5'
AdThd (30 µM) alone did not affect the radiosensitivity of
647V cells. These results further suggest that the enhanced radiosens-
itzing effect of the IdUrd-5'-AdThd combination is due to the
increased incorporation of IdUrd in the DNA of 647V cells.

In order to test the specificity of the 5'-AdThd response, we
screened 3 other cell lines for the effect of 5'-AdThd on IdUrd
incorporation into DNA (Fig. 5). T24, another invasive human
bladder cancer cell line, and SV-HUC-TT1, a tumorigenic cell
line derived from SV-HUC, demonstrated similar 5'-AdThd-
mediated increases in IdUrd incorporation. However, SV-
HUC, a prototype normal human urothelial cell line, did not
exhibit any stimulation of IdUrd incorporation into DNA.
Thus, the 5'-AdThd-mediated increase in IdUrd replacement of
dThd in DNA is apparently specific for these human bladder
cancer cell lines.

It appears that the effect of 5'-AdThd on IdUrd-induced radio-
sensitivity in 647V cells is due to its antagonization of
dTTP-mediated feedback inhibition of TK. The intracellular
concentration of dTTP in 647V cells has been measured at 24
µM (11). In purified enzyme preparations, TK would be com-
pletely inhibited by such a high dTTP concentration. Also,
while 5'-AdThd inhibited IdUrd incorporation into the DNA
of SV-HUC cells (Fig. 5), IdUrd cellular uptake in SV-HUC
was stimulated as reported in a previous study (11).

These observations suggest at least three possible explana-
tions. First, triphosphate pools may be compartmentalized (33)
allowing TK to function at a level close to that seen in its
purified state. This would also permit high IdUTP concentra-
tions if TK was present in sufficient quantities to maintain a
saturating IdUTP concentration for incorporation into DNA.
However, incorporation data presented here for SV-HUC and previously published uptake data in SV-HUC cells (11) suggest that stimulating the uptake of IdUrd alone is not sufficient to increase IdUrd incorporation in the DNA of a cell. Therefore, other mechanisms may regulate IdUrd incorporation into DNA.

Second, TK may also be under a different type of regulation in situ. While the purified TK from normal human urothelial and mouse C3H/10T1/2 cells showed a response similar to TK derived from 647V with respect to 5'-AdThd stimulation, only a marginal increase in dThd uptake was demonstrated in these cells lines (11). It has also been reported that there exists a pH differential between normal and tumor tissues (34, 35) with tumor pH generally more acidic. The strong pH dependence on dTTP inhibition of TK (36) suggests that this type of regulation may be involved. These alternative regulatory mechanisms may be critical since a number of anticancer and antiviral drugs are activated by TK.

Third, there is also the possibility of secondary sites of action for 5'-AdThd. Under conditions of low pH (6.8) in 647V cells, we recently reported increased radiosensitivity when cells were exposed to 2 μM IdUrd plus 300 μM 5'-AdThd (37). While this result corresponds with purified enzyme and cellular uptake data for 647V, IdUrd incorporation was inhibited under these conditions. This suggests that 5'-AdThd in high concentration with cells incubated at pH 6.8 has a second site of action.

It is also interesting to note that the level of incorporation of 2 μM IdUrd into DNA is less in 647V (7.9%) and SV-HUC-TT1 (5.7%) than in either T24 (11%) or SV-HUC cells (11.1%). This may result in a lower initial level of IdUrd-mediated DNA damage on 647V and SV-HUC-TT1 versus T24 and SV-HUC cells. However, cotreatment with IdUrd plus 5'-AdThd should specifically increase IdUrd incorporation in the tumor lines, resulting in an increase in the overall therapeutic gain, as demonstrated in 647V cells.

5'-AdThd represents an example of a nontoxic agent capable of specifically altering the regulation of a key enzyme involved in the activation of antitumor drugs. We conclude that 5'-AdThd is capable of specifically increasing the uptake and incorporation of IdUrd into the DNA of bladder cancer cell lines at clinically relevant IdUrd concentrations with a resulting increase in the radiosensitivity of at least one of these cell lines. The combination of IdUrd and 5'-AdThd to selectively enhance radiosensitization is currently undergoing testing in vivo using these human bladder cancer xenografts.

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