ABSTRACT

The feasibility of noninvasive imaging of adenoviral-mediated herpes virus thymidine kinase (HSV1-tk) gene transfer and expression was assessed in a well-studied animal model of metastatic colon carcinoma of the liver. Tumors were produced in syngeneic BALB/c mice by intrahepatic injection of colon carcinoma cells (MCA-26). Seven days later, three different doses ($3 \times 10^8$, $1 \times 10^8$, and $3 \times 10^7$ plaque-forming units (pfu)) of the recombinant adenoviral vector ADV. Rous sarcoma virus (RSV)-tk bearing the HSV1-tk gene were administered by intratumoral injection in separate groups of mice. Two control groups of tumor-bearing mice received intratumoral injections of the control adenoviral vector dl-312 or buffer alone, respectively. T2-weighted magnetic resonance (MR) images of mice were obtained before administering the virus and provided an anatomical reference of hepatic tumor localization. Eighteen h after the virus injection, one group of animals was given i.v. injections of 300 µCi of no-carrier-added $5^{\text{131}}$I-2*fluoro-1-$\beta$-D-arabinofuranosyl-uracil (FIAU) and imaged 24 h later with a gamma camera. In some animals, the tumors were sampled and processed for histology and quantitative autoradiography (QAR). The gamma camera images demonstrated highly specific localization of $[^{131}\text{I}]$FIAU-derived radioactivity to the area of ADV.RSV-tk-injected tumors in the liver, which was confirmed by coregistering the gamma camera and T2-weighted MR images. There was no accumulation of $[^{131}\text{I}]$FIAU-derived radioactivity in tumors that were injected with the control vector or injection solution alone. A more precise distribution of radioactivity in the area of transfected tumors was obtained by histological and QAR comparisons. A heterogeneous pattern of radioactivity distribution in transfected tumors was observed. A punctate pattern of radioactivity distribution was observed in peritumoral liver tissue in animals given injections of $3 \times 10^8$ and $1 \times 10^8$ pfu of ADV.RSV-tk but not in animals given injections of $3 \times 10^7$ pfu nor in control animals. A QAR-microscopic comparison showed that the punctate areas of radioactivity colocalized with cholangial ducts. The level of $[^{131}\text{I}]$FIAU-derived radioactivity accumulation (HSV1-tk expression) in the transplanted tumors was viral dose-dependent. The viral dose-dependency of radioactivity accumulation was more pronounced in peritumoral liver, which was confirmed by reverse transcription-PCR analysis. A separate group of tumor-bearing animals received different doses of ADV.RSV-tk vector followed by treatment with ganciclovir (GCV), 10 mg/kg i.p. b.i.d. for 6 days. The ADV.RSV-tk transplanted tumors significantly regressed with GCV treatment; the control tumors continued to grow. During the GCV treatment, the levels of liver transaminases (ALT and AST) were significantly increased in animals that received injections of $3 \times 10^8$ and $1 \times 10^8$ pfu of ADV.RSV-tk but not in animals that received injections of $3 \times 10^7$ pfu and in control animals. The observed liver toxicity confirms the results of gamma camera and QAR imaging, which demonstrated an unwanted spread of ADV.RSV-tk vector and HSV1-tk expression in peritumoral and remote liver tissue at higher doses. These and our previous results indicate that noninvasive imaging of adenoviral-mediated HSV1-tk gene expression is feasible for monitoring cancer gene therapy in patients.

INTRODUCTION

A limiting factor in the application and optimization of clinical gene therapy protocols is the inability to monitor the delivery and expression of therapeutic genes in target tissues. Noninvasive imaging of transgene expression would be of considerable value in many ongoing and future clinical gene therapy trials by defining the location, magnitude, and persistence of transgene expression over time. In our initial studies, a paradigm for noninvasive imaging of transgene expression was described and validated (1–3).

Briefly, this paradigm requires the appropriate combination of a “marker gene” and “marker substrate.” The marker gene product (an enzyme) converts the marker substrate to a metabolite that is selectively trapped within the transduced cell. The paradigm is essentially an in vivo radiotracer enzyme assay. The feasibility and implementation of the paradigm was demonstrated using the HSV1-tk$^3$ gene as a marker gene and radiolabeled FIAU as a marker substrate.

FIAU crosses the cell membrane by the diffusion and facilitated transport (thymidine transporters) mechanisms. In HSV1-tk-transduced tumor cells, the HSV1-tk enzyme phosphorylates FIAU to FIAU monophosphate (FIAU-MP), which does not cross the cell membrane and accumulates inside the cells. Thus, radiolabeled FIAU-derived radioactivity accumulates in transduced but not in nontransduced tumor cells. In transduced tumor cells which do not proliferate (quiescent cells), the FIAU-MP accumulates mainly in the cytoplasm. In transduced proliferating cells, FIAU-MP can be phosphorylated by the endogenous cellular di- and triphosphate kinases to FIAU-DP and FIAU-TP and integrated into the DNA by DNA-polymerase. As the radiolabeled FIAU levels in blood and tissues decrease due to the urinary excretion, the retained FIAU-derived radioactivity in HSV1-tk-transduced tumor tissues becomes distinguishable from low background tissue radioactivity (2).

We have demonstrated that highly specific images of HSV1-tk gene expression in experimental animal tumors after retroviral-mediated HSV1-tk gene transfer can be obtained using radioiodinated $[^{131}\text{I}]$FIAU and a clinical gamma camera system or QAR(2). We also demonstrated that a significant increase in sensitivity and resolution of images could be achieved with $[^{131}\text{I}]$FIAU and a clinical PET system (3). Moreover, we demonstrated that a quantitative relationship exists between FIAU accumulation and independent measures of HSV1-tk gene expression (1, 3). All of our previous studies were performed using retroviral-mediated gene transfer (low efficacy of transduction) to explore the lower limit of sensitivity of the described imaging technology.

Among the commonly used gene transfer vectors, Ad vectors are...
particularly attractive for in vivo gene therapy of cancer (for review, see Refs. 4–6). Several previous studies have shown that Ad-vectors, in contrast to retroviral vectors, are capable of transferring and expressing different genes not only in proliferating cells, but also in quiescent cells. In addition, Ad-vectors can be produced in large quantities at high titers and large doses of vector can be administered. Despite the relatively high efficiency of Ad-vectors in delivering genes to tumor cells in vivo, expressing those genes at sufficient levels in target tissue to achieve the optimal therapeutic efficacy remains problematic (7, 8). Although, there have been a number of studies that evaluated different promoters for optimal gene expression from Ad-vectors in vitro (9–11), few studies have been designed to optimize the Ad-vector-mediated transgene expression in normal tissues (12) and none in preexisting tumors in vivo.

In the current study, we assessed the feasibility and sensitivity of imaging of HSV1-tk gene transfer and expression after in vivo Ad-mediated gene transfer to hepatic tumors in a well-studied animal model of MCA-26 metastatic colon carcinoma of the liver (13, 14). Imaging HSV1-tk expression was performed with [131I]FIAU and a clinical gamma camera and by QAR. The sensitivity of gamma camera imaging of different levels of HSV1-tk gene expression was assessed after intratumoral administration of different doses of the Ad vector. HSV1-tk expression in transplanted tumor tissue and surrounding liver was also assayed independently by RT-PCR. Ad-vector dose-dependent liver toxicity after GCV therapy was also assessed by measuring the activity of the aminotransferases in serum. The gamma camera and QAR images of HSV1-tk expression in transplanted tumor tissue and surrounding liver were compared with these independent measures of gene expression.

MATERIALS AND METHODS

Cell Cultures. The MCA-26 mouse colon carcinoma is a chemically induced, poorly differentiated colon carcinoma cell line derived from BALB/c mice (15). The MCA-26 cells were obtained from American Type Tissue Culture Collection and grown as monolayers in a composite medium containing 50% DMEM and 50% F-12 medium with 10% FCS and 10 mM HEPES (pH 7.3). Human 293 cells (embryonic kidney cells) were immortalized with the early region of the Ad serotype 5 and grown as monolayers in DMEM with 10% FCS (16). The gp-SPK-A2 vector producer cell line, which produces the recombinant replication-deficient STK retrovirus containing the NeoR gene and HSV1-tk gene was described previously (17). The gp-SPK-A2 cells were grown as monolayers in DMEM with 10% FCS. The MCA-26 cells were transduced in vitro with the STK retroviral vector (MCA26TK+ cells) as described previously (1). The MCA26TK+ cells, as well as the single-cell-derived clone MCA26TK435, were grown in MEM with 10% FCS and 250 µg/ml G418. The cells were released from the culture plates by treatment with 0.5% trypsin in PBS for 5–10 min, resuspended in the growth media to neutralize the trypsin and passed. To produce a cell suspension for injection, cells were centrifuged and a cellular pellet was obtained and resuspended in MEM (without FCS) at a concentration of 10⁷ viable cells in 100 µl.

Ad Vectors. Construction of a replication-defective, E1- and E3-deleted Ad vector (based on human Ad serotype 5 containing the HSV1-tk gene under transcriptional control of the RSV enhancer/promoter (ADV.RSV-tk) has been reported previously (18, 19). The HSV1-tk-negative Ad vector Ad.dl-312, harboring a deletion in the E1 region, was a gift from Dr. Tom Shenk (Princeton, University, Princeton, NJ). The viruses were amplified in human 293 cells and purified as described previously (20). The viral titer was measured by plaque assay in human 293 cells.

Hepatic and s.c. Tumors. The experimental protocol involving animals was approved by the Institutional Animal Care and Use Committee of the Memorial Sloan Kettering Cancer Center. Liver tumors were produced in syngeneic BALB/c mice (Harlan Sprague Dawley, Indianapolis, IN). The laparotomy was performed under anesthesia (Ketamine, 87 mg/kg, and Xylazine, 13 mg/kg, i.p.) and the MCA-26 cells (5 × 10⁶ cells in 10 µl) injected at the tip of the left lateral liver lobe as described previously (13). s.c. tumors in anesthetized mice were produced by s.c. injection of MCA26 or MCA26TK#35 cells (10⁶ cells in 100 µl).

MRI Studies. MRI was performed on a Bruker Omega CSI system (Bruker, Fremont, CA) with a 4.7 Tesla, 33-cm bore magnet equipped with shielded gradients and a custom-designed solenoid resonator as described previously (21). Briefly, the mice were anesthetized with the mixture of Ketamine 87 mg/kg and Xylazine 13 mg/kg, i.p. and placed in the radiofrequency coil; the mice were positioned with their livers at the center of the solenoid resonator. Series of coronal T2-weighted 1-mm thick images were obtained using a 256 × 128 matrix, 80-mm field of view, and standard spin-echo sequences (TR = 3500 msec, TE = 40 msec, and 2 excitation phase encoding step). The MRI studies were performed 1 day before radiotracer injection. Liver tumors were detected as the areas of hyperintense T2 signal. The MR images provided the anatomical reference of hepatic tumor localization and were used to interpret gamma camera images.

No-Carrier-added Synthesis [131I]FIAU. No-carrier-added [131I]FIAU was prepared by reacting 2′-fluoro-2′-deoxy-1-b-D-arabinofuranosyl-uracil (FAU, Moravek Biochemicals, Brea, CA) with carrier-free 131I using iodogen followed by isolation of the product by column chromatography as described previously (2). Briefly, the [131I]FIAU injection solution was prepared by evaporation of the methanol-eluted fraction from the C18 cartridge and dissolving the residue in a sterile pyrogen-free physiological saline solution and by passage through a sterile nonpyrogenic 0.22 µm Millipore filter. Radiochemical purity of [131I]FIAU was assessed using HPLC system consisting of a HPXL Pump (Rainin, Woburn, MA), Flo-One Beta detector Series 100 (Radiomatic, Meriden, CT). Data were collected and analyzed using online Dynamax software (Rainin, Woburn, MA). HPLC conditions included the following: (a) a 250 × 4.6 mm reverse phase 10-µm C18 Maxsil column (Phenomenex, Torrance, CA); (b) the isotactic mobile phase of 10% methanol in water; and (c) a flow rate of 1.5 ml/min. The results of radio-HPLC analysis of the [131I]FIAU synthesis yielded a >95% pure, pyrogen-free compound. The theoretical specific activity of [131I]FIAU/produced by a no-carrier-added synthesis was calculated (3).

Gamma Camera Imaging. No-carrier-added [131I]FIAU (300 µCi per animal) was injected i.v. One day before [131I]FIAU administration, all of the animals received an i.p. injection of 0.9% NaCl solution (1 ml) to block the thyroid uptake of radioactive iodide. Gamma camera imaging was performed on a dual-headed ADAC Vertex gamma camera (ADAC, Milpitas, CA) equipped with a high-energy high-resolution (HEHR) collimator. Planar images were obtained and reconstructed to a 512 × 512 matrix with 1.2 × 1.2-mm pixel size.

QAR. After the gamma camera imaging session, mice were killed by cervical dislocation. The s.c. and hepatic tumors were extracted rapidly, frozen, and processed for QAR and histology as described previously (2). Tissue sections were exposed to the SB-5 film (Kodak, Rochester, NY) along with a set of autoradiographic standards of known 131I concentration, freshly prepared using the liver homogenate. Knowing the tissue radioactivity and the injected dose, we converted the autoradiographic images to parametric images of %dose/g tissue and color coded to a range of values. The H&E-stained tissue sections were digitized and coregistered to the corresponding autoradiographic images for precise localization of accumulated radioactivity. This was performed on a digital image analysis system using MCID software (Imaging Research, Hamilton, Ontario, Canada). The computerized morphometric analysis of the largest cross-sectional areas was performed on H&E-stained tumor sections.

To estimate organ dosimetry of radiiodinated [131I]FIAU and to confirm the validity of the gamma camera measurements of radioactivity, the tissue samples were weighed and directly assayed for radioactivity using a Packard 5500 gamma spectrometer (Packard, Meriden, CT).

RT-PCR Analysis. RT-PCRs were carried out to determine the HSV1-tk gene expression in the tumor and surrounding liver tissues. Total RNA was isolated from the tumor and liver tissues, using the Ultraspec RNA isolation system (Biotex, Houston, TX). For the first cDNA synthesis, the SuperScript preamplification System (Life Technologies, Inc.) was used in combination with a HSV1-tk specific primer and 2.0 µg of total RNA as template, according to the protocol provided by supplier. Then 10% of the first strand cDNA obtained were amplified directly using PCR with a pair of HSV1-tk specific primers. The sense primer was 5′-CTCCACCTCATCTTGGACGGC-3′, and the antisense primer was 5′-CCTGCAATACCGCACCCT-3′. This set of
primers amplifies a 290-bp segment of HSV1-tk coding sequence. PCR was performed in a DNA thermal cycler (Perkin-Elmer) with a Taq DNA polymerase, using the following profile: (a) 25 cycles of denaturing at 94°C for 1 min; (b) primer annealing at 55°C for 30 s; and (c) extension at 72°C for 1 min. RT-PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide.

**Serum Biochemistry.** Biochemical parameters of hepatic injury were evaluated by serum assays for ALT and AST using a Kodak Ektachem 700 analyzer using standard manufacturer’s procedures.

**Experimental Groups.** Four sets of studies were performed. The first set of experiments was performed to test the feasibility of gamma camera imaging of HSV1-tk with [131I]FIAU in mice. One wild-type MCA-26 tumor and one transduced MCA26TK#35 clonal tumor were produced after s.c. injections of the cell lines in mice (in the dorsal parts of the left and right thighs, respectively). The HSV1-tk-transduced MCA26TK#35 tumors and wild-type MCA26 tumors served as positive and negative controls, respectively.

The second set of experiments was performed to assess the feasibility and sensitivity of imaging Ad-mediated transfer and expression of HSV1-tk in transplanted tumor and surrounding liver tissue in mice. Imaging [131I]FIAU uptake and retention was performed with a clinical gamma camera and QAR.

Two wild-type MAC26 tumors were produced in each animal: one intrahepatic and one s.c. located in the dorsal part of the right thigh. Seven days later, the mice were anesthetized again (as described above); a laparotomy was performed, and the recombinant Ad vector ADV.RSV-tk was injected directly into the 4 × 5-mm hepatic tumors (in 50 μl of 10% TRIS-HCl (pH 7.4)/1 mM MgCl₂/10% (vol/vol) glycerol/Polybrene, 20 μg/ml) as a single injection into the center of the tumor (needle 30 Ga), administered slowly over 5 min in 10 μl/min increment boluses. After the injection, the needle was left in the tumor for another 5 min to prevent reflux of the inoculum and to ensure its resorption. The ADV.RSV-tk virus was administered at different doses: 3 × 10⁶; 1 × 10⁶; and 3 × 10⁵ pfu/50 μl (three animals per dose). A wild-type s.c. MCA26 tumor in the thigh served as a negative control. Two additional control groups of similar tumor-bearing mice received intratumoral (hepatic) injections of 3 × 10⁶ of a control (non-HSV1-tk-containing) Ad vector Ad.ddl-312 (n = 7) or buffer alone (n = 7), respectively.

The third set of experiments was performed to assess Ad-mediated transfer and expression of HSV1-tk in transplanted tumor and surrounding liver tissue by RT-PCR analysis. The hepatic tumor-bearing mice (n = 9) were treated with 3.0 × 10⁶ pfu of the ADV.RSV-tk vector; no GCV was administered. The mice were killed at days 2, 5, and 8 after vector administration. Total RNA was extracted from tumor and surrounding liver tissues and was subjected to RT-PCR analysis. The results of these experiments were compared with those obtained in the second set (gamma camera and QAR imaging).

The fourth set of studies was performed to evaluate viral dose-dependent antitumor and hepatotoxic effects of ADV.RSV-tk-pls-GCV combination therapy, and to determine an appropriate dose of ADV.RSV-tk vector with maximum therapeutic efficacy and minimum hepatotoxicity. In this set, one intrahepatic wild-type MCA26 tumor was produced in each animal. At day 7, when the tumor size reached about 4 × 5 mm², the ADV.RSV-tk virus was administered at different doses: 5 × 10⁶; 3 × 10⁵; 1 × 10⁵; and 3 × 10⁴ pfu (8 animals for each dose) as described above. Control groups of mice received similar doses of Ad.ddl-312 Ad vector. Beginning 12 h after the viral injections, the mice were treated i.p. with GCV at 10 mg/kg b.i.d. for five consecutive days. To monitor the hepatic toxicity of Ad administration combined with GCV treatment, blood samples were collected weekly, and the serum levels of ALT and AST were monitored for 3 weeks. The results of these studies were compared with those obtained in previous sets of experiments.

**RESULTS**

**Imaging HSV1-tk Expression with [131I]FIAU and Gamma Camera or QAR.** In the first set of experiments, highly specific gamma camera images of [131I]-radioactivity accumulation and retention were obtained in s.c. MCA26TK#35 tumors at 24 h after the administration of [131I]FIAU (Fig. 1). The average level of radioactivity retention in MCA26TK#35 clonal tumors was high (2.6 ± 0.2% dose/g). In contrast, the wild-type MCA26 tumors showed substantially less retention of radioactivity (<0.01% dose/g), almost at background levels.

In the second set of experiments, the MR images clearly demonstrated the localization of hepatic tumors in the frontal edge of the left lobe of the liver (Fig. 2). The gamma camera images were obtained at 24 h after administration of [131I]FIAU and revealed highly specific accumulations of [131I]FIAU-derived radioactivity in the areas of MCA26TK#35 tumors.
of intrahepatic MCA26 tumors that were transfected with the ADV.RSV-tk vector (Fig. 3) and identified by MRI (Fig. 2). The average level of radioactivity retention assessed by gamma camera imaging was: (a) highest in MCA-26 intrahepatic tumors transfected with $3 \times 10^8$ pfu of the ADV.RSV-tk vector (Fig. 3A); (b) medium in tumors transfected with $1 \times 10^7$ pfu (Fig. 3B); and (c) slightly lower in tumors transfected with $3 \times 10^7$ pfu (Fig. 3C). In contrast, the wild-type MCA26 tumors showed insignificant retention of radioactivity that was at the background levels (Fig. 3). Similar low (background) levels of $[\text{I}^{131}\text{I}]$FIAU-derived radioactivity were observed in the areas of intrahepatic tumors in two additional control groups of mice that received an intratumoral (hepatic) injection of the control (non-HSV1-tk-bearing) Ad vector Ad.dl-312 or buffer alone (images not shown). Increased levels of peritumoral liver radioactivity were observed in animals injected with $3 \times 10^8$ and $1 \times 10^8$ pfu of the ADV.RSV-tk vector (Fig. 3, A and B) but not in animals that received $3 \times 10^7$ pfu (Fig. 3C).

QAR images of intrahepatic tumor and surrounding liver tissue revealed a heterogeneous distribution of $[\text{I}^{131}\text{I}]$FIAU-derived radioactivity. The average level of radioactivity retention was highest in MCA-26 intrahepatic tumors transfected with $3 \times 10^8$ pfu of the ADV.RSV-tk vector (1.6 ± 0.4% dose/g; Fig. 4, A and B). In these animals, a punctate heterogeneous pattern of $[\text{I}^{131}\text{I}]$FIAU-derived radioactivity was observed in the surrounding liver tissue. When the QAR images were coregistered with the corresponding H&E-stained tissue sections, it became apparent that this punctate pattern colocalized with the small biliary ducts and subcapsular spaces of the liver. A distinct gradient of radioactivity distribution was observed in peritumoral liver tissue (Fig. 4, A, B, and C). The average level of liver radioactivity in these animals was 0.026 ± 0.006% dose/g. A similar heterogeneous pattern of $[\text{I}^{131}\text{I}]$FIAU-derived radioactivity distribution was observed in tumors transfected with $1 \times 10^8$ pfu of the ADV.RSV-tk vector, whereas remote liver tissue showed much less punctate foci of radioactivity (Fig. 4C). The average level of radioactivity retention in transfected MCA-26 tumors was slightly lower (1.1 ± 0.3% dose/g; Fig. 4, C and D), whereas the average level of liver radioactivity in these animals was substantially lower (0.009 ± 0.002% dose/g). Tumors transfected with $3 \times 10^7$ pfu of the ADV.RSV-tk vector showed a slightly lower level of radioactivity retention (0.8 ± 0.2% dose/g; Fig. 4, E and F), whereas only a few punctate foci of $[\text{I}^{131}\text{I}]$FIAU-derived radioactivity were observed in peritumoral liver tissue, and the average level of liver radioactivity in these animals was very low (0.004 ± 0.001% dose/g), in the range of background activity (Table 1). The wild-type MCA26 tumors and tumors injected with the control Ad vector Ad.dl-312 or buffer alone showed very low levels of radioactivity retention at 24 h (<0.005% dose/g; images not shown); the average level of liver radioactivity in these animals was also very low (0.003 ± 0.001% dose/g) and similar to that observed in animals treated with $3 \times 10^7$ pfu of the ADV.RSV-tk vector. The levels of $[\text{I}^{131}\text{I}]$FIAU-derived radioactivity measured by γ spectroscopy in tissue samples are summarized in Table 1.

A linear relationship was observed between the viral dose (pfu) and the level of $[\text{I}^{131}\text{I}]$FIAU-derived radioactivity accumulated in tumors and peritumoral liver (Fig. 5). The level of $[\text{I}^{131}\text{I}]$FIAU-derived radioactivity accumulated in tumors increased by a factor of two with a 10-fold increase of vector dose from $3 \times 10^7$ to $3 \times 10^8$ pfu. In contrast, the level of $[\text{I}^{131}\text{I}]$FIAU-derived radioactivity in peritumoral liver increased roughly by a factor of 6 to 7.

HSV1-tk Expression in the Tumor and Surrounding Liver. In the third set of experiments, transfection of MCA-26 intrahepatic tumors with $3 \times 10^8$ pfu of the ADV.RSV-tk vector resulted in levels of HSV1-tk mRNA expression that were moderately high in the transfected tumor, lower in the same liver lobe, and lowest in another liver lobe, throughout the time points. In control animals treated with Ad.dl-312, no specific HSV1-tk mRNA was detected (Fig. 6).

Viral Dose-dependent Effects of ADV.RSV-tk and GCV Combination Therapy. In the control group of animals treated with $3 \times 10^8$ pfu of Ad.dl-312, the mean viable tumor cross-section was 27.5 ± 12 mm² with minimal central necrosis. In mice that received different doses of ADV.RSV-tk vector followed by GCV therapy (10 mg/kg b.i.d. for 5 days) an inverse relationship was observed between the maximum cross-sectional areas of residual viable tumors and the doses of ADV.RSV-tk vector (Fig. 7). One of 8 animals treated with $3 \times 10^8$ pfu of ADV.RSV-tk vector and GCV had a complete tumor regression. In mice treated with different doses of ADV.RSV-tk, the serum levels of ALT and AST enzymes during GCV treatment remained relatively normal until the doses reached 1.0–3.0 × 10⁸ pfu (Fig. 8). At the dose of 5 × 10⁸ pfu or higher, however, the enzyme levels were significantly elevated, and most of the animals in this group died 1 or 2 weeks later. The maximum tolerable dose of ADV.RSV-tk was 3.0 × 10⁸ pfu and is consistent with that reported previously by Chen et al. (13). The animals treated with 3.0 × 10⁸ pfu of the control Ad.dl-312 vector and GCV had serum ALT and AST levels within the normal range.
DISCUSSION

In the current study, we demonstrated that noninvasive imaging of Ad-mediated HSV1-tk gene transfer and expression in a mouse model of colorectal carcinoma metastasis to the liver is feasible with \([^{131}I]\)FIAU and a clinical gamma camera system. As we demonstrated previously, a significant increase in sensitivity and resolution of images could be achieved with \([^{124}I]\)FIAU and a clinical PET system (3), and a quantitative relationship exists between radioiodinated FIAU accumulation and independent measures of HSV1-tk gene expression (1, 3). Recently, Gambhir et al. (22) demonstrated that imaging of Ad-mediated HSV1-tk gene transfer and expression in

<table>
<thead>
<tr>
<th>Tissue</th>
<th>3 × 10^8 pfu</th>
<th>1 × 10^8 pfu</th>
<th>3 × 10^7 pfu</th>
<th>Ad.dl-312</th>
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<td></td>
<td>(n = 3)</td>
<td>(n = 3)</td>
<td>(n = 3)</td>
<td>(n = 14)</td>
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<tr>
<td>Tumor(e)</td>
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<td>0.006 ± 0.004</td>
<td>0.005 ± 0.003</td>
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<tr>
<td>Plasma</td>
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<td>0.051 ± 0.022</td>
<td>0.046 ± 0.015</td>
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</table>

\(d\) The data are the group averages ± SD.
\(e\) \(n\), number of animals.
\(d\) The levels are calculated from the combined data obtained in mice that received Ad.dl-312 (\(n = 7\)) or buffer alone (\(n = 7\)).
\(d\) Intrahepatic MCA-26 tumors transfected with ADV.RSV-tk vector.
\(d\) Subcutaneous nontransfected control MCA-26 tumors.

 previously, a significant increase in sensitivity and resolution of images could be achieved with \([^{124}I]\)FIAU and a clinical PET system (3), and a quantitative relationship exists between radioiodinated FIAU accumulation and independent measures of HSV1-tk gene expression (1, 3). Recently, Gambhir et al. (22) demonstrated that imaging of Ad-mediated HSV1-tk gene transfer and expression in
mouse liver after i.v. administration of an Ad vector is feasible with radiolabeled [8-14C]GCV and QAR or with [8-18F]fluoroganciclovir and PET (22, 23). It was demonstrated that i.v. administration of the Ad vector results primarily in transfection of the liver. A good correlation was also demonstrated between the [8-14C]GCV % dose/g and HSV1-TK enzyme levels in HSV1-tk positive tissues (22, 23). The same group also reported on the synthesis of [131I]-labeled penciclovir and demonstrated that 18F-labeled penciclovir had better imaging properties (about 2-fold greater accumulation) than [18F]-labeled GCV for imaging HSV1-tk expression (24).

In this study, we used the ADV.RSV-tk Ad vector, in which the expression of HSV1-tk gene is under control of the RSV enhancer/promoter element. The RSV promoter is not the most potent as compared with other promoters that are currently used in gene therapy vector design (e.g., the cytomegalovirus promoter). However, the levels of HSV1-tk expression achieved in transfected intrahepatic tumors were adequate for noninvasive imaging with [131I]FI AU using a clinical gamma camera or QAR. The in vivo transfection of tumors could be clearly visualized on gamma camera images, and a more precise localization of transfected tumors was achieved by comparing the gamma camera images with T2 MR images obtained in the several axial planes. High resolution QAR images revealed a heterogeneous pattern of [131I]FIAU accumulation in transfected tumor tissues, which suggests an uneven spread of vector after intratumoral injection. The average level of [131I]FIAU accumulation in transfected intrahepatic MCA-26 tumors correlated weakly with the dose (pfu) of injected ADV.RSV-tk vector. Although statistically significant (r = 0.99), a 10-fold increase in vector dose from 3 × 107 to 3 × 108 pfu resulted in only a 2-fold increase in [131I]FIAU accumulation (HSV1-tk expression) in transfected tumors. In contrast, a 10-fold increase in vector dose from 3 × 107 to 3 × 108 pfu resulted in a significant improvement of response to GCV therapy that manifested as a significant decrease in tumor size in the animals that received 3 × 108 pfu.

The apparent disparity between the 2 fold increase in [131I]FIAU accumulation (HSV1-tk expression) in transfected tumors and significant improvement of response to GCV therapy observed with the increase of vector dose from 3 × 107 to 3 × 108 pfu may be explained by an increased bystander effect from the transfected peritumoral liver tissue that expressed relatively high levels of HSV1-tk after intratumoral injection of 3 × 108 pfu of ADV.RSV-tk vector. This hypothesis is supported by the observation that the direct intratumoral injection of high doses of Ad vector (1 × 108 and 3 × 109 pfu), even in a small volume of injectate (50 µl), can result in a significant peritumoral spread of the Ad vector and in transfection of peritumoral and remote liver tissues. Gamma camera images demonstrated the increased levels of [131I]FIAU-derived radioactivity in peritumoral liver at doses of 3 × 108 and 1 × 109 pfu/tumor of the ADV.RSV-tk vector. More detailed images of radioactivity distribution in the tumors were obtained with QAR and revealed a punctate heterogeneous pattern of [131I]FIAU-derived radioactivity distribution in peritumoral and remote liver tissue in mice that received doses of 3 × 108 and 1 × 109 pfu/tumor, but minimal or no punctate areas were seen at a dose of 3 × 107 pfu/tumor. This punctate pattern of radioactivity distribution was shown to colocalize with small bile ducts. This suggests that, after intratumoral injection, the vector spreads into the remote liver tissue via the cholangial system and that the cholangial
epithelium of small biliary ducts can be transfected with this Ad vector. The epithelium of large bile ducts did not accumulate any radioactivity, which suggests that transfection is limited to the small biliary duct system at this dose of vector.

The average level of $^{[131]}$I]FIAU accumulation in peritumoral and remote liver tissue was 2-log orders lower than that of transfected tumors, but the level of uptake was more highly correlated with the dose (pfu) of the ADV.RSV-tk vector. ALT and AST levels were measured at different times after the initiation of therapy with GCV (10 mg/kg, b.i.d.).

In conclusion, noninvasive imaging of Ad-mediated HSV1-tk gene transfer and expression is feasible with $^{[131]}$I]FIAU and a clinical gamma camera system. Nevertheless, a significant increase in sensitivity and resolution of images can be achieved with $^{[124]}$I]FIAU and a clinical PET. Imaging HSV1-tk expression after Ad-mediated intratumoral gene transfer could be used for the assessment of the efficacy of gene transfer and expression in the ongoing clinical gene therapy trials. It could aid in the assessment of vector specificity and safety by providing information (whole body images) about the location, magnitude, and duration of HSV1-tk gene expression over time. Moreover, it could aid in dose optimization of new Ad vectors and in establishing the optimal dose and timing of GCV treatment.

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Imaging Adenoviral-mediated Herpes Virus Thymidine Kinase Gene Transfer and Expression  In Vivo

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