Myocardial Kinetics of Reporter Probe ^{124}I-FIAU in Isolated Perfused Rat Hearts After In Vivo Adenoviral Transfer of Herpes Simplex Virus Type 1 Thymidine Kinase Reporter Gene

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Reporter gene imaging holds promise for noninvasive monitoring of cardiac gene therapy. We recently demonstrated that ^{124}I-labeled 2′-fluoro-2′-deoxy-5′-iodo-1β-D-arabinofuranosyluracil (^{124}I-FIAU) is suitable for PET of myocardial expression of herpes simplex virus type 1 thymidine kinase reporter gene (HSV1-tk). In contrast to previous studies in tumors, early specific uptake was followed by rapid washout. Myocardial kinetics of ^{124}I-FIAU are still poorly understood. This study aimed at a further investigation under controlled conditions using an isolated heart perfusion model. **Methods:** Male Wistar rats underwent transthoracic regional injection of replication-defective adenovirus (2.5×10^9 plaque-forming units) containing either HSV1-tk ($n = 16$) or LacZ reporter gene ($n = 15$) into the inferior wall. Nonmanipulated rats ($n = 5$) served as further controls. Hearts were excised 2 d later and perfused according to the Langendorff technique with ^{124}I-FIAU–containing buffer (15 min, followed by 30 min of nonradioactive perfusion). Experiments were performed under baseline conditions and in the presence of thymidine (competitive substrate) or fludarabine (in vitro inhibitor of 5′-nucleotidase). Time–activity curves were acquired by external coincident detectors. The myocardial rate of ^{124}I-FIAU uptake (K_i), clearance rate (K_o), and volume of distribution ($V_d = K_i/K_o$) were calculated. Subsequently, hearts were subjected to γ-counting, followed by microtome slicing and autoradiography. **Results:** The V_d from Langendorff perfusion significantly correlated with final whole-heart tracer retention ($r = 0.88$, $P = 0.019$) and the autoradiographic area of regional myocardial activity ($r = 0.89$, $P = 0.016$). HSV1-tk hearts showed higher K_i and V_d of ^{124}I-FIAU compared with that of controls ($P < 0.001$) and detectable but slower washout compared with that of the LacZ group ($P < 0.01$). Addition of thymidine to the perfusate inhibited myocardial uptake of ^{124}I-FIAU by reducing V_d and K_i in HSV1-tk and LacZ hearts compared with the baseline. Addition of fludarabine did not result in the expected reduction of washout in HSV1-tk hearts due to inhibition of 5′-nucleotidases (which may dephosphorylate ^{124}I-FIAU monophosphate). It acted as an uptake inhibitor similar to thymidine, reducing V_d in HSV1-tk hearts. **Conclusion:** Assessment of specific reporter probe kinetics after regional in vivo reporter gene transfer is feasible using the isolated perfused rat heart preparation. This model allows one to study the effects of pharmacologic interventions and may refine understanding of the reporter probe signal for in vivo imaging. Different nucleoside analogs significantly inhibit ^{124}I-FIAU uptake, emphasizing the importance of transporter mechanisms for reporter probe kinetics.

Key Words: radioisotopes; reporter genes; herpes simplex virus type 1 thymidine kinase reporter gene; gene therapy

J Nucl Med 2005; 46:98–105

Cardiac gene transfer is a promising therapeutic strategy for several diseases, such as ischemic heart disease and cardiac failure (*1,2*). There is increasing interest in the development of imaging strategies that allow for noninvasive evaluation of the localization and extent of transgene expression in the heart over time (*3,4*). The principle of nuclear imaging of transgene expression is based on reporter genes expressing a gene product that is normally absent in host tissue and that results in specific accumulation of a radioactive reporter probe (*5*).

Recently, herpes simplex virus type 1 thymidine kinase gene (HSV1-tk) has been described as a reporter gene (*6,7*). Among various compounds, the radiolabeled nucleoside analog 2′-fluoro-2′-deoxy-5′-iodo-1β-D-arabinofuranosyluracil (FIAU) is a suitable substrate, with high sensitivity and selectivity for detection of HSV1-tk expression (*8*).

In contrast to results obtained in tumors, which demonstrated an increased and stable uptake of radiolabeled FIAU (*9*), a recent in vivo study addressing gene imaging in heart

Received Feb. 26, 2004; revision accepted Aug. 30, 2004.

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tissue reported washout of ^{124}I-FIAU after initial specific uptake in HSV1-tk–transduced tissue (*10*). This seems to be a specific feature of myocardial tissue, and the underlying mechanisms remain poorly understood.

The isolated perfused heart preparation has been demonstrated to be useful for assessment of global myocardial tracer kinetics (*11*). This approach has the advantage that interfering systemic factors, such as tracer degradation, are avoided, allowing for more specific and controlled studies. However, the feasibility of this method for quantitative assessment of reporter probe accumulation after regional myocardial gene transfer has not yet been evaluated.

Thus, this investigation aimed at (a) testing the feasibility of isolated perfused hearts as a model for quantification of myocardial gene expression after in vivo regional gene transfer; and (b) investigating the mechanisms involved in myocardial washout of FIAU by using specific pharmacologic interventions during the perfusion experiments.

MATERIALS AND METHODS

Male Wistar rats weighing ~250 g were studied. Animals were fed normal rodent diet with free access to tap water. The study protocol was approved by the regional governmental commission for animal protection (Regierung von Oberbayern).

In Vivo Myocardial Gene Transfer

Animals were anesthetized with intramuscular injection of midazolam (0.1 mg/kg), fentanyl (1 μg/kg), and medetomidine (10 μg/kg). Intramyocardial injection of adenoviral vectors into the inferior left ventricular wall was performed percutaneously from the epigastric angle under continuous echocardiographic guidance using a constant injection volume of 150 μL.

Experimental groups were constituted according to the viral vector injected. The HSV1-tk group underwent intramyocardial injection of 2.5×10^9 plaque-forming units (pfu) of a replication-defective adenoviral vector, type 5, carrying HSV1-tk complementary DNA under the transcriptional control of human cytomegalovirus early gene promoter ($n = 16$). The LacZ group received similar adenovirus carrying the LacZ gene under control of the same promoter and served as a negative control ($n = 15$). Nonmanipulated normal rats served as further controls ($n = 5$).

Isolated Heart Perfusion

Two days after local adenovirus injection, isolated heart perfusion preparation was performed according to the Langendorff technique, as previously described (*12*). Briefly, animals were euthanized under anesthesia (intraperitoneal injection of pentobarbital, 65 mg/kg), and hearts were rapidly harvested, placed in ice-chilled buffer, and immediately mounted via ascending aorta cannulation on a retrograde perfusion system at constant flow rate of 12 mL/g of heart weight/min. Perfusion solution was a modified Krebs–Henseleit bicarbonate buffer (pH 7.4), warmed to 37°C, oxygenated with 95% O_2/5% CO_2, and containing (in mmol/L) NaCl, 117; KCl, 4.7; $MgSO_4$, 1.1; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 20; and glucose, 10.

A water-filled latex balloon was inserted in the left ventricle, and the developed left ventricular systolic pressure (cm H_2O) and heart rate were continuously recorded. After a 10-min stabilization phase, the perfusate was switched to a buffer containing ^{124}I-FIAU (74 kBq/mL) for 15 min (wash-in phase). This was followed by a further 30-min perfusion with nonradioactive normal buffer (wash-out phase).

^{124}I-FIAU was synthetized as previously described (*10*). Myocardial uptake of ^{124}I-FIAU was measured by 2 external coincidence detectors interfaced with a computer, and time–activity curves were generated (*13*). Acquired data were corrected for decay of radioactivity and background activity and normalized to heart weight and to buffer radioactivity concentration.

For analysis of the wash-in phase, equilibration of tracer in interstitial and vascular spaces within the first 2 min was assumed (*11*). Uptake thereafter reflects specific accumulation of ^{124}I-FIAU in the intracellular compartment. To estimate the total myocardial uptake rate of ^{124}I-FIAU, K_i (mL/g/min), we compared the results of regression analysis using 2 curve-fitting options, power and linear. Both regressions yielded similar correlation coefficient values: linear, 0.88 ± 0.04; and power, 0.88 ± 0.04 ($P = 0.36$, paired t test). Because the application and interpretation of linear regression fit are straightforward, we considered using linear fit as a good option for further kinetic data analysis. Thus, the K_i was estimated from the slope of the linear regression of the time–activity curve during this wash-in phase.

In the ^{124}I-FIAU wash-out curve, the initial 2-min period is considered to be related to nonspecific tracer clearance from interstitial and vascular compartments. The following slower clearance phase reflects specific tracer washout from the cellular compartment (*13*). The best method to describe the ^{124}I-FIAU clearance kinetics was investigated by comparing the regression analysis using linear and monoexponential curve fitting. The monoexponential fitting rendered a higher correlation coefficient (0.95 ± 0.016) than that obtained with linear regression (0.92 ± 0.02; $P = 0.0062$). Thus, the constant E from the fitted monoexponential function $y = A \cdot \exp(E \cdot t)$ was considered to estimate the clearance rate for tracer originating from cellular spaces (K_o). Figure 1 illustrates the fitted curves applied on time–activity curves obtained from an HSV1-tk heart. Finally, the volume of distribution, V_d (mL/g), was obtained by the K_i/K_o ratio.

Pharmacologic Interventions

Hearts from all experimental groups were perfused at baseline condition (HSV1-tk, $n = 5$; LacZ, $n = 5$; no intervention, $n = 5$). The effect of specific drugs, which were added to the perfusion buffer for the entire perfusion time (wash-in and wash-out phases), was tested in HSV1-tk hearts ($n = 6$) and compared with LacZ hearts as controls ($n = 5$).

Fludarabine phosphate (F-Ara-AMP) is a fluorinated nucleotide analog of the antiviral agent vidarabine and has been reported to inhibit cytosolic 5′-nucleotidase in cell culture (*14*). 5′-Nucleotidase is an enzyme that is highly abundant in myocardium and dephosphorylates nucleotides such as FIAU monophosphate, a product of HSV1-tk. F-Ara-AMP was added at a dose of 10 μmol/L to the perfusate for 10 hearts (HSV1-tk, $n = 5$; LacZ, $n = 5$). This dosage was chosen based on previous in vitro studies showing that F-Ara-AMP inhibits the 5′-nucleotidase activity by a competitive mechanism (*14*). That study used an F-Ara-AMP concentration that was 5-fold higher than the concentration of the natural substrate thymidine, obtaining a 75% inhibition of the enzyme activity. Because in vivo studies investigating the inhibitory properties of F-Ara-AMP are missing, we applied a concentration 10^6 higher than the concentration of the radioactive sub-

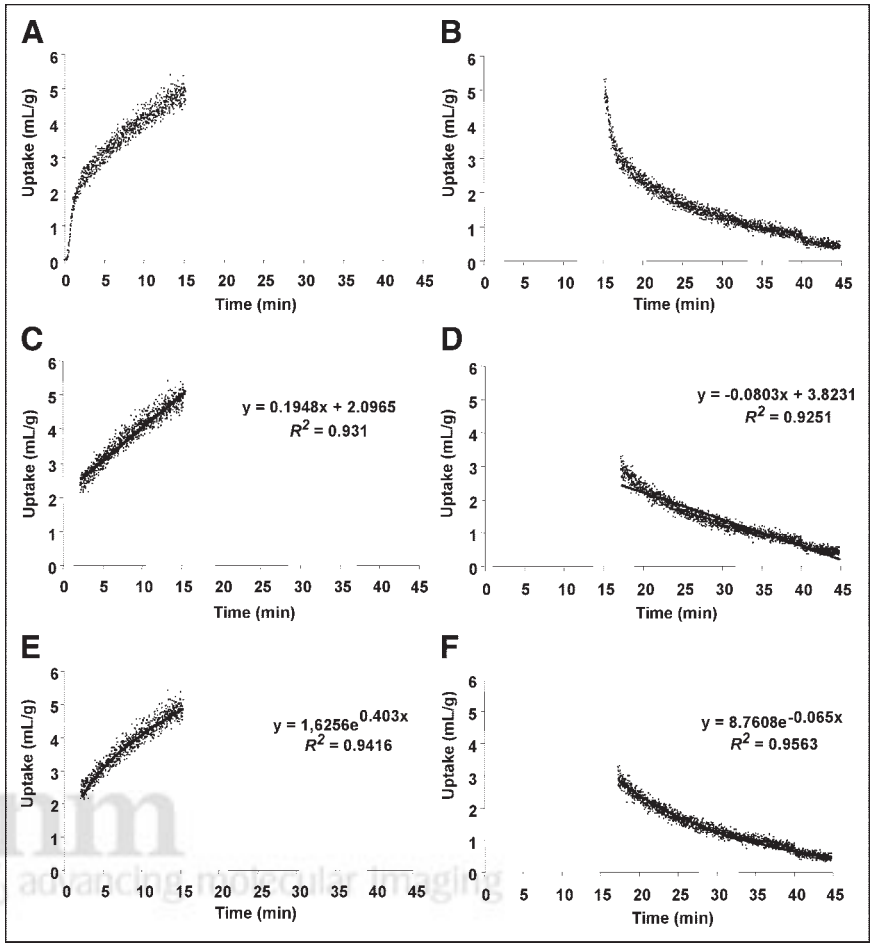

FIGURE 1. Examples of curve fitting applied on time–activity curves obtained from HSV1-tk rat heart. Whole time–activity curve was split into wash-in (A) and wash-out (B) components. (C and E) Linear and power fitting for wash-in curve phase, respectively. (D and F) Linear and monoexponential fitting applied on wash-out curve phase.

strate (^{124}I-FIAU, 12.5 pmol/L) with the intention to ensure an enzyme inhibition effect.

Thymidine is a natural substrate of all thymidine kinase enzymes and was added to the perfusate at a dose of 10 μmol/L as a competitive inhibitor of ^{124}I-FIAU uptake in 10 hearts (HSV1-tk, $n = 5$; LacZ, $n = 5$). This dosage was chosen following the same approach as that used for F-Ara-AMP to get an excess of the competitive substrate for a profound inhibitory effect on radioactive substrate uptake.

γ-Counting

After perfusion, hearts were rinsed in saline and excess liquid was removed. After weighing, whole hearts were counted in a well γ-counter (Cobra Quantum; Packard Instruments Co.). Counts were corrected for background activity and for radioactivity decay. A retention index of radioactivity in the whole heart, RI (mL/g), was obtained through the ratio between radioactivity concentration in the heart (counts/g) and radioactivity concentration in the buffer (counts/mL).

Autoradiography

After γ-counting, hearts were rapidly frozen. Short-axis slices (20 μm) were prepared (HM 500 OM microtome; Microm) and digital autoradiography was obtained (PhosphorImager 445 SI; Molecular Dynamics). Analysis of the presence and the extent of regional myocardial tracer retention was performed. Count density images were normalized to the maximum pixel count, and a threshold of 20% was applied. An area of the viral vector injection exhibiting increased tracer retention was then manually drawn in each of the cardiac slices. The slices exhibiting the largest increased tracer uptake areas were then identified. In these slices, the percentage area of the tracer accumulation in relation to the total slice area was then obtained. This value was used as an index estimating the percentage myocardial area of increased tracer uptake.

Statistical Analysis

Results are expressed as mean ± SEM. One-way ANOVA and the multiple comparisons Tukey–Kramer post hoc test were used to identify differences between the means of the 3 experimental groups. The Student *t* test was used to evaluate individual pairs of values of independent variables, and the Student paired *t* test was used for dependent variables. To test for correlation between variables, linear regression was used. P values < 0.05 were considered significant.

RESULTS

Baseline Kinetics

Baseline kinetic data from isolated perfused HSV1-tk hearts show a higher myocardial ^{124}I-FIAU uptake rate compared with those of LacZ and control hearts (Table 1). Additionally, the clearance rate was correspondingly re-

TABLE 1
Baseline Myocardial ^{124}I-FIAU Kinetic Data

Parameter	Experimental group			*P* (ANOVA)
	HSV1-tk	LacZ	Control	
Uptake rate				
K_i (mL/g/min)	0.19 ± 0.02	0.08 ± 0.01*	0.09 ± 0.01*	0.0003
Clearance rate				
K_o (mL/g/min)	0.04 ± 0.003	0.07 ± 0.01†	0.05 ± 0.0003	0.01
Volume of distribution				
V_d (mL/g)	4.90 ± 0.98	1.29 ± 0.21†	1.84 ± 0.27†	0.0022

*$P < 0.001$ vs. HSV1-tk.
†$P < 0.01$ vs. HSV1-tk (Tukey–Kramer post hoc test).

duced in HSV1-tk hearts compared with that of LacZ hearts. These changes resulted in an increased V_d in HSV1-tk hearts compared with the V_d of the 2 other experimental groups (Fig. 2). The clearance rate of control hearts was very similar to that observed in the HSV1-tk group. This finding indirectly suggests that the clearance rate of LacZ hearts was increased compared with that of the control group, even though no statistically significant difference was found.

Effects of Pharmacologic Interventions

HSV1-tk Hearts. With the addition of F-Ara-AMP to the perfusion buffer, HSV1-tk hearts presented a significant reduction of the ^{124}I-FIAU uptake rate while no significant change of tracer clearance rate was observed (Figs. 3A and 3C; Table 2). These changes resulted in a 47% reduction of the V_d compared with the baseline value (Fig. 4; Table 2). With addition of thymidine to the perfusion buffer, a more pronounced inhibition of the ^{124}I-FIAU uptake rate was demonstrated in HSV1-tk hearts (Figs. 3A and 3E), resulting in an 83% decrease of the V_d (Fig. 4; Table 2).

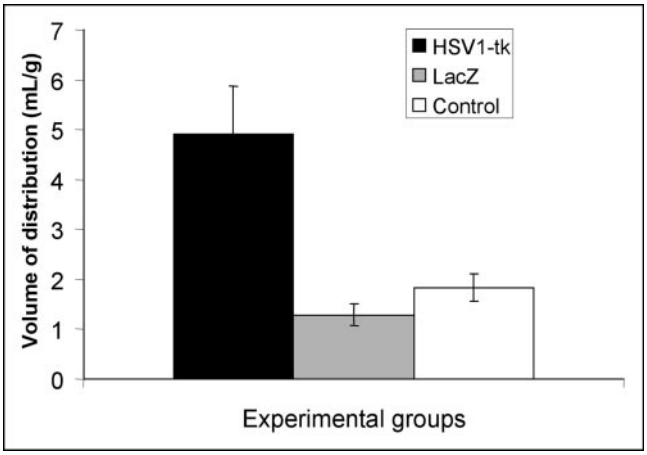

FIGURE 2. Baseline V_d for ^{124}I-FIAU in isolated perfused hearts of 3 experimental groups ($P < 0.01$ vs. other groups). Detailed mean data are displayed in Table 1.

LacZ Hearts. In contrast to results obtained with HSV1-tk hearts, in the LacZ hearts addition of F-Ara-AMP produced only a slight reduction of the uptake rate (Figs. 3B and 3D) and no change of the clearance rate. The V_d demonstrated no significant reduction compared with baseline (Fig. 4; Table 2). On the other hand, the presence of thymidine in the perfusion buffer caused a marked reduction of 95% of the V_d of ^{124}I-FIAU (Figs. 3B and 3F; Table 2).

Additionally, the ^{124}I-FIAU uptake rate in the presence of thymidine was higher in the HSV1-tk group (0.04 ± 0.01 mL/g/min) compared with that of the LacZ hearts (0.005 ± 0.003 mL/g/min, $P = 0.0018$). This suggests a higher affinity of HSV1-tk for the radiolabeled nucleoside analog FIAU than for the natural substrate thymidine.

Model Validation

The RI assessed by γ-counting after the end of isolated perfusion demonstrated higher values in HSV1-tk hearts (0.67 ± 0.1) compared with those in LacZ (0.35 ± 0.04; $P < 0.05$ vs. HSV1-tk) and control groups (0.17 ± 0.04; $P < 0.01$ vs. HSV1-tk). Autoradiographic images of HSV1-tk hearts showed large areas of increased tracer uptake in the injection sites involving 36% ± 3% of the total myocardial slice area. No areas with regional ^{124}I-FIAU accumulation were identified in LacZ or control hearts (Fig. 5). We reported previously that the area of increased ^{124}I-FIAU uptake is related to the area of positive thymidine kinase immunohistochemical staining (*5*).

The V_d determined by isolated perfusion showed a significant positive correlation with the RI measured by use of γ-counting in the 16 HSV1-tk hearts ($P = 0.019$, $r = 0.88$; Fig. 6). Also, a significant correlation was found between the V_d and the percentage myocardial area exhibiting ^{124}I-FIAU accumulation in the 6 HSV1-tk hearts submitted to perfusion under the baseline condition, the only ones in which this analysis was feasible because of the presence of areas of increased tracer uptake ($P = 0.016$, $r = 0.89$; Fig. 7).

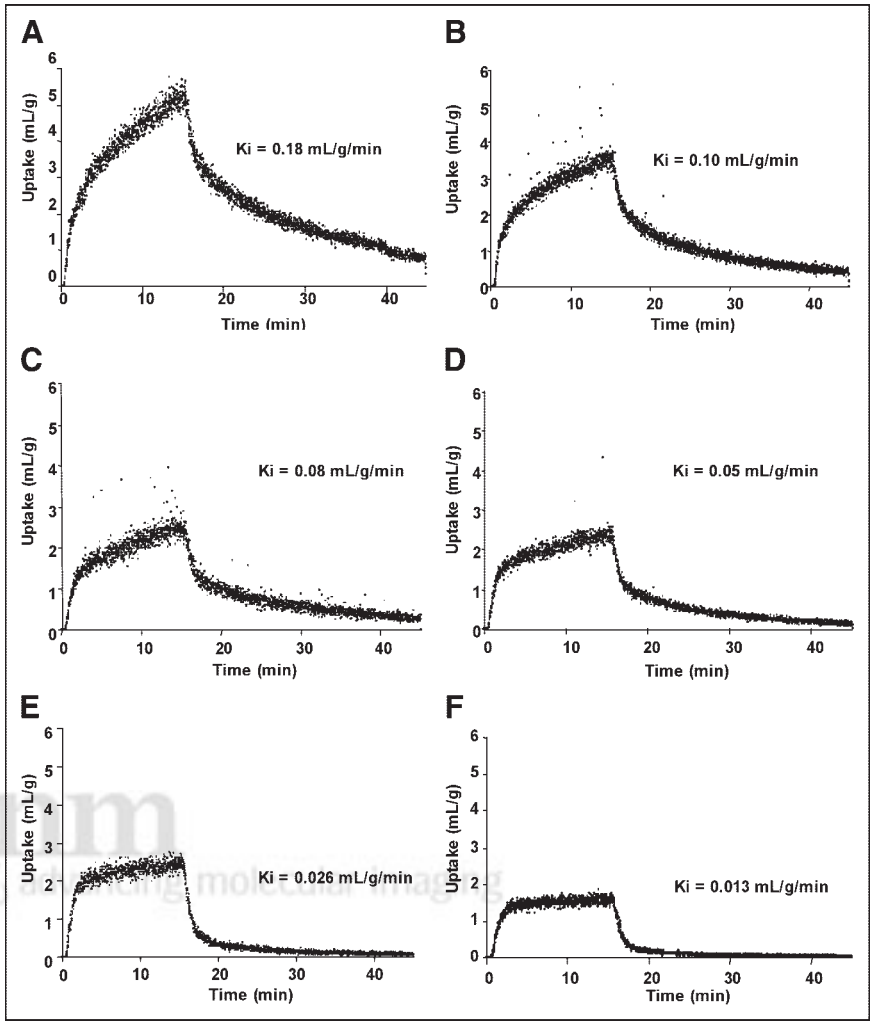

FIGURE 3. Examples of representative time–activity curves obtained in isolated perfused HSV1-tk hearts (left) and LacZ hearts (right) at baseline (A and B), under effect of F-Ara-AMP (C and D), and under effect of thymidine (E and F), respectively.

DISCUSSION

The use of FIAU as a reporter probe for detecting expression of HSV1-tk was first introduced in oncology. Previous studies in HSV1-tk transduced tumors suggested that FIAU is phosphorylated intracellularly by a reporter gene product and then incorporated into host DNA (*15*). Kinetic analysis showed that FIAU uptake increased rapidly within the first hour and then remained stable until 24 h after injection. However, we recently observed that the in vivo kinetics of FIAU in HSV1-tk transduced pig myocardium

TABLE 2
Effects of Pharmacologic Interventions on Kinetics of Myocardial ^{124}I-FIAU in HSV1-tk and LacZ Hearts

Parameter	Baseline	F-Ara-AMP	Thymidine	*P* (ANOVA)
HSV1-tk				
K_i (mL/g/min)	0.19 ± 0.02	0.11 ± 0.01*	0.04 ± 0.01†	<0.0001
K_o (mL/g/min)	0.04 ± 0.004	0.05 ± 0.002	0.05 ± 0.01	0.0426
V_d (mL/g)	4.90 ± 0.98	2.09 ± 0.19*	0.83 ± 0.22†	0.0007
LacZ				
K_i (mL/g/min)	0.08 ± 0.01	0.05 ± 0.004‡	0.005 ± 0.003†	<0.0001
K_o (mL/g/min)	0.07 ± 0.001	0.06 ± 0.003	0.08 ± 0.004	0.0649
V_d (mL/g)	1.29 ± 0.21	0.85 ± 0.08	0.06 ± 0.04†	<0.0001

*$P < 0.01$ vs. HSV1-tk.
†$P < 0.001$ vs. baseline.
‡$P < 0.05$ (Tukey–Kramer post hoc test).

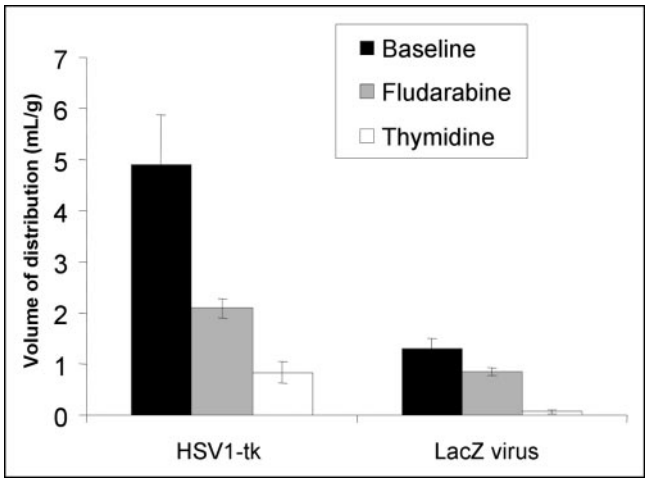

FIGURE 4. Effect of pharmacologic interventions on V_d in HSV1-tk and LacZ hearts. Significant reduction of V_d is seen in both groups under effect of F-Ara-AMP and thymidine ($P < 0.01$ for comparison with baseline condition). Detailed mean data are displayed in Table 2.

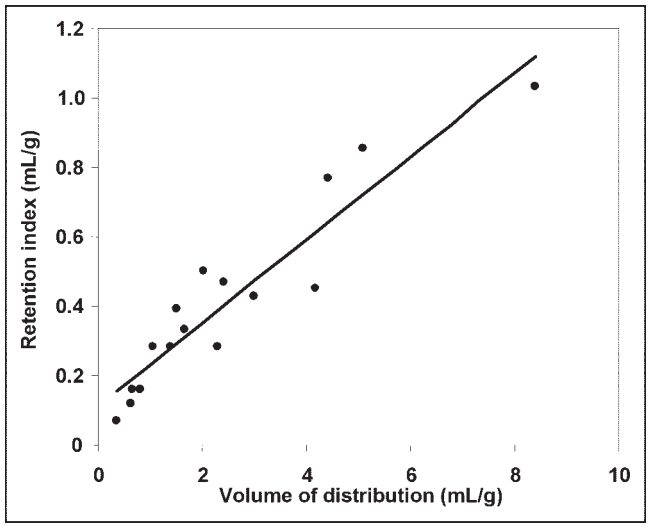

FIGURE 6. Scatter plot illustrates positive correlation between perfusion-derived V_d and γ-counter–derived RI in HSV1-tk hearts investigated under baseline and pharmacologic interventions ($r = 0.913$, $P < 0.0001$).

were different from those reported in HSV1-tk transduced tumors. Early specific uptake was followed by continuous washout (*10*). Because radioactivity efflux after initial uptake was not observed in tumors, tissue-specific differences in nucleoside metabolism, which influence reporter probe kinetics, were assumed.

The present data in isolated hearts confirm these previous observations but allow for a more detailed analysis of underlying mechanisms. Similar to observations in living pigs, continuous cardiac tracer clearance was demonstrated after specific initial uptake. Systemic degradation of tracer in noncardiac tissue and blood, which may result in limited tracer delivery in the living organism, is absent in the isolated heart preparation. Thus, FIAU washout from HSV1-tk transduced heart represents an intrinsic myocardial mechanism and is not a result of extrinsic factors that reduce tracer influx over time (*10*).

Isolated heart perfusion proved to be a feasible method for quantitative assessment of reporter probe kinetics after myocardial gene transfer in this study. ^{124}I-FIAU accumulation derived from kinetic data correlated well with static ex vivo indices estimating tracer retention. Positive correlations were found at baseline and in the presence of high doses of the natural substrate thymidine, which acted as a competitive inhibitor of tracer uptake. Moreover, thymidine inhibited ^{124}I-FIAU accumulation in HSV1-tk transduced hearts to a lesser degree than that in control hearts, supporting the higher specificity of ^{124}I-FIAU for HSV1-tk compared with endogenous thymidine kinases.

In addition, the results of the present study show only a modest increase of myocardial ^{124}I-FIAU uptake rate (2.4-

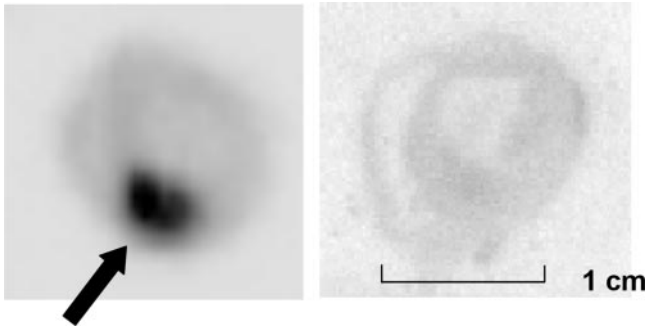

FIGURE 5. Autoradiography of representative myocardial short-axis slices. (A) HSV1-tk heart: enhanced radioactivity accumulation is seen in injection site (arrow) (left ventricular inferior wall). (B) LacZ heart: no regional tracer retention is detected.

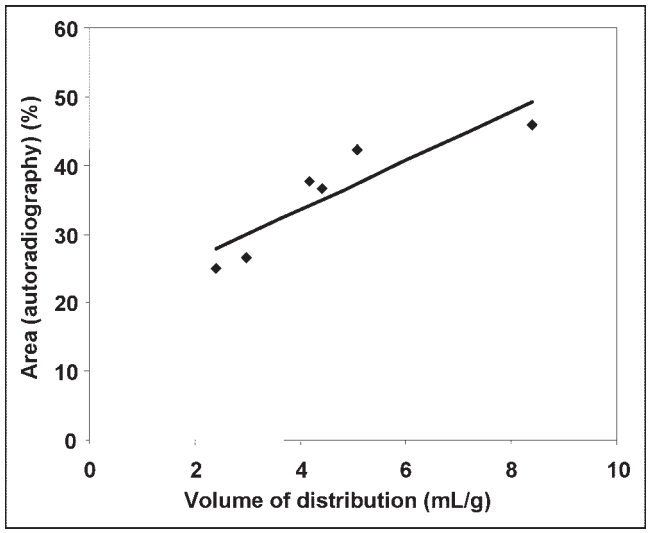

FIGURE 7. Scatter plot demonstrates positive correlation between perfusion-derived baseline V_d and autoradiography-derived index estimating myocardial area exhibiting increased ^{124}I-FIAU uptake in HSV1-tk hearts ($r = 0.89$, $P = 0.016$).

fold) in HSV1-tk hearts compared with that of the control hearts. This indirectly indicates a reduced ratio of the target organ to background activity in an in vivo imaging scenario and represents a possible limitation of using this tracer for in vivo myocardial imaging of HSV1-tk gene expression in a clinical context.

Because of the low tissue proliferation rates in myocardium compared with those of tumors, DNA turnover, and thus DNA incorporation of nucleotides (phosphorylated nucleosides), is expected to be low. This may facilitate intracellular degradation and tracer efflux. Specific enzymatic mechanisms exist, which oppose the effect of kinases and avoid the accumulation of nucleotides that can be cytotoxic (*16*). Those nucleotidase enzymes are present in the cytosol and mitochondria. For example, cytosolic 5′-nucleotidase is expressed at high levels in the myocardium and is thought to be involved in production of adenosine, a potent vasodilator and a cardioprotective substance (*17*). Nucleotidases may thus account for intramyocardial dephosphorylation of phosphorylated FIAU and subsequent tracer washout. We sought to test this hypothesis under controlled conditions in the isolated perfused heart but were limited by the fact that agents that selectively inhibit nucleotidases in vivo have not yet been clearly identified.

Nucleotide and nucleoside analogs have been described as inhibitors of the cytosolic 5′-nucleotidase-I in heart (*18*). The nucleotide analog F-Ara-AMP has been described as a nucleotidase inhibitor in previous in vitro experiments (*14*). After cell entrance, F-Ara-AMP is rapidly submitted to dephosphorylation by the action of membrane and cytosolic 5′-nucleotidase (*19*). However, since F-Ara-AMP appears to be a poor substrate for cytosolic nucleotidase, it works as a competitive inhibitor of the activity of this enzyme (*14*). We applied this substance in our perfusion model but observed that it was inhibitory rather than stimulating on ^{124}I-FIAU accumulation. These results can be interpreted in different ways: If F-Ara-AMP selectively inhibited nucleotidases in our setting, those may not be the primary mechanism responsible for ^{124}I-FIAU washout. More likely, however, F-Ara-AMP may have exerted additional effects in the in vivo setting that go beyond in vitro described nucleotidase inhibition, albeit limiting its usefulness to study the role of nucleotidases in vivo. The present data suggest some competition between F-Ara-AMP and ^{124}I-FIAU for steps involved in ^{124}I-FIAU accumulation outweighing the inhibitory effects on nucleotidases. For example, those may be HSV1-tk enzyme itself or nucleoside transport into myocytes (*20*).

Specific transport proteins in the plasma membrane mediate the uptake of nucleoside into cells because they are relatively hydrophilic molecules (*21*). Nucleoside transporters play a key role for the regulation of many physiologic processes, such as adenosine uptake and extracellular concentration, and for the salvage pathway of nucleic acid precursors (*20,22*). Two types of transporters have been described, the equilibrative facilitated-diffusion type and the concentrative sodium-dependent type (*20*). Equilibrative transporters are widely distributed in different cell types and tissues and have been identified also in myocardium (*22,23*). The equilibrative transporters, in contrast to the concentrative type, present broad substrate specificities for pyrimidine and purine nucleosides. Both nucleosides used in this study, F-Ara-AMP (purine nucleoside) and thymidine (pyrimidine nucleoside), can serve as the substrate for equilibrative transporters and compete with the pyrimidine nucleoside ^{124}I-FIAU, causing reduction in the uptake.

To further refine the understanding of the apparently complex kinetics of radiolabeled nucleoside reporter probes, detailed studies to dissect processes of transport, phosphorylation, dephosphorylation, and DNA incorporation will be necessary along with identification and characterization of agents suitable for specific pharmacologic intervention. In this regard, an analysis of the effluent buffer after myocardial perfusion by HPLC could be used to investigate the concentration of phosphorylated and degraded FIAU content and contribute to the understanding of the wash-out mechanism.

Improved understanding of the kinetics of reporter probes is desirable because reporter gene imaging holds great promise for monitoring of specific cardiovascular molecular interventions. The recent progress achieved by gene transfer therapy has emphasized the need for in vivo techniques for quantitative assessment of the gene expression time course as a critical parameter for gene delivery evaluation and therapy monitoring (*2,24*). The combination of HSV1-tk reporter gene and radiolabeled nucleosides as reporter substrate is, at present, the best-investigated approach for cardiac molecular–genetic imaging. Detailed information about nucleoside metabolism and factors interfering with the tissue uptake of the radiolabeled compounds will contribute to the development of future clinical applications of this methodology.

Some limitations of the present study need to be emphasized. In this study we assessed the counts emitted by the whole heart to estimate the kinetics of ^{124}I-FIAU in myocardial regions expressing the HSV1-tk gene. However, the total counts emitted by the whole heart included also those originating from other myocardial regions not expressing the HSV1-tk gene and from the intracavitary, intravascular, and extracellular spaces. We recognize that these factors could have influenced the kinetic parameter estimates. However, we consider that these factors are also present in the other experimental groups and the main conclusion based on the differences observed between the different experimental groups can be held. On the other hand, more complex modeling could have been used to correct for these factors and achieve more precise estimates of kinetic parameters. However, this was beyond the scope of this study, which was designed to be a first step in the investigation of ^{124}I-FIAU myocardial kinetics based on an isolated heart preparation.

CONCLUSION

Isolated heart perfusion is a feasible method for quantitative assessment of reporter probe kinetics after myocardial reporter gene transfer at baseline and during specific pharmacologic interventions. Addition of thymidine to the perfusate confirmed its role as a natural substrate and an inhibitor of ^{124}I-FIAU accumulation. In vivo effects of F-Ara-AMP, a substance that acts as a nucleotidase inhibitor in vitro, were similar to the effect of thymidine in reducing ^{124}I-FIAU accumulation. This result suggests a competitive inhibition on a common metabolic pathway step, most probably at the level of the membrane transporter, emphasizing the importance of transporter mechanisms for the kinetics of reporter probes. Metabolic pathways of nucleoside reporter probes in the heart require further investigations in the future. For those, the controlled conditions of the isolated perfused rat heart may serve as a reliable model.

ACKNOWLEDGMENTS

This study was supported by research grants from Deutsche Forschungsgemeinschaft (Be 2217/4-1), from the Kommission für klinische Forschung, Klinikum rechts der Isar (KKF 8764167), and from Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (01/04868-8).

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