Uptake of Radiolabeled 2′-Fluoro-2′-Deoxy-5-Iodo-1-β-D-Arabinofuranosyluracil in Cardiac Cells After Adenoviral Transfer of the Herpesvirus Thymidine Kinase Gene

The Cellular Basis for Cardiac Gene Imaging

Frank M. Bengel, MD; Martina Anton, PhD; Norbert Avril, MD; Thomas Brill, MD, DVM; Ngoc Nguyen, BSc; Roland Haubner, PhD; Elisabeth Gleiter, PhD; Bernd Gansbacher, MD; Markus Schwaiger, MD

Background—Gene therapy is a promising approach for the treatment of cardiac diseases. Coexpression of therapeutic genes with a suitable marker gene would allow for the noninvasive imaging of successful gene transfer and expression via radiolabeled marker substrates. In the present study, such an approach was first applied to cardiac tissue.

Methods and Results—The combination of the herpesvirus thymidine kinase reporter gene (HSV1-tk) and radiolabeled 2′-fluoro-2′-deoxy-5-iodo-1-β-D-arabinofuranosyluracil (FIAU) was evaluated. H9c2 rat cardiomyoblasts were infected in vitro with a replication-defective HSV1-tk–containing adenovirus and a negative control virus. The intracellular uptake of [14C]FIAU increased with increasing multiplicity of infection and with time after infection. Uptake in negative controls remained <15% of positive controls. Additionally, vectors were applied intramyocardially in Wistar rats. The marker substrate [125I]FIAU was injected intravenously 3 days later, and animals were killed after 24 hours. Autoradiographically, regional transgene expression was clearly identified in animals receiving the adenovirus containing HSV1-tk (3.4±2.2-fold increase of radioactivity at vector administration site compared with remote myocardium), whereas nonspecific uptake in negative controls was low (<10% of positive controls).

Conclusions—Using an adenoviral vector, HSV1-tk can be successfully expressed in cardiac cells in vitro and in vivo, yielding high uptake of radiolabeled FIAU. The results suggest that imaging transgene expression in the heart is feasible and may be used to monitor gene therapy noninvasively. (Circulation. 2000;102:948-950.)

Key Words: gene therapy • imaging • genes, reporter • arabinofuranosyluracil • radioisotopes

Myocardial gene therapy is rapidly evolving and holds promise for the treatment of diseases such as heart failure and ischemia.1 Recently, clinical phase I trials for the treatment of ischemia using an adenovirus expressing vascular endothelial growth factor cDNA have been conducted.2 The success of cardiac gene delivery is currently monitored by indirect measures, such as symptomatic improvement or clinical test results, and the direct assessment of gene expression is feasible ex vivo only. Therefore, a noninvasive, clinically applicable method for imaging successful myocardial gene transfer is of considerable value. Monitoring gene therapy would be facilitated by defining the localization, extent, magnitude, and persistence of gene expression over time.

Recently, the herpes simplex virus type 1 thymidine kinase gene (HSV1-tk) was used as a marker gene.3,4 HSV1-tk is normally not present in host tissue, and it encodes for an enzyme catalyzing phosphorylation and, thus, intracellular accumulation of marker substrates. Among various substrates, radiolabeled 2′-fluoro-2′-deoxy-5-iodo-1-β-D-arabinofuranosyluracil (FIAU) demonstrated high sensitivity and selectivity for the detection of HSV1-tk expression.5 FIAU is trapped intracellularly only in the presence of HSV1-tk (Figure 1). This tracer has been used successfully for imaging HSV1-tk expression in retrovirally transduced tumors in vivo.6 Ultimately, the marker gene can be coexpressed with effector genes for the subsequent imaging of therapeutic gene transfer. This concept, however, has not yet been applied to the heart.

Thus, the aim of the present study was to determine the feasibility of HSV1-tk, transduced by an adenoviral vector, and radiolabeled FIAU as a marker gene/marker substrate for imaging transgene expression in the heart.

Methods

In Vitro Assessment of FIAU Uptake

A replication-defective adenoviral vector type 5 (AdHCMV-TK) carrying herpes simplex virus thymidine kinase cDNA under the
transcriptional control of human cytomegalovirus (HCMV) early gene promoter was constructed according to standard methods. The rat cardiomyoblast cell line H9c2 was studied, and the human prostate cancer cell line DU145 served as a “positive” control for the comparison of transduction efficiency and time course of gene expression. Cells were seeded in 6-cm dishes at 1x10^5 cells/dish and incubated in Dulbecco’s modified essential medium plus 10% fetal calf serum.

To determine the relationship between vector concentration and FIAU uptake, cells were infected with increasing multiplicities of infection (MOIs; 0, 10, 25 and 50) of AdHCMV-TK or Addl70–3^9 (used as a negative control). At 24 hours after infection, the medium was replaced by 5 mL of medium containing 2-[^14]C]-FIAU (55 mCi/mL) and methyl-[^3]H]-thymidine (TdR; 65 Ci/mmol) (Hartmann Analytic). Concentrations were 0.01 μCi/mL for [^14]C]-FIAU and 0.2 μCi/mL for [^3]H]-TdR. After incubation for 1 hour, the medium was removed and cells were washed, scraped, resuspended, and transferred to scintillation vials containing 1 mL of tissue solubilizer (Soluene-350, Packard). After 12 hours, scintillation fluid (Lumasafe Plus, Lumac LSC) was added. Samples were measured in a Win Spectral 1414 liquid scintillation counter (Wallac) by dual-channel counting. Uptake of [^14]C]-FIAU and [^3]H]-TdR was expressed as a percent of total tracer in medium. [^14]C]-FIAU accumulation was then corrected for cell proliferation by normalization to [^3]H]-TdR uptake.

To determine the time course of FIAU accumulation after gene transfer, both cell lines were infected with AdHCMV-TK at MOI 10. [^14]C]-FIAU- and [^3]H]-TdR-containing media were given at 6, 12, 24, 48, 72, 96, and 120 hours after infection. Tracer uptake was measured as described above. Uptake was also measured in negative (mock-infected) control dishes after 48 and 96 hours. Generally, each experiment was conducted twice and repeated in case of a difference >10%. Results are reported as the average of 2 values.

### Intramyocardial Application In Vivo and Autoradiography

Under anesthesia, 6 male Wistar rats received an intramyocardial injection of 2.3x10^5 plaque-forming units of either AdHCMV-TK (n=3) or a negative control adenovirus (AdHCMV-LacZ; n=3) into the inferior wall. Injection was performed percutaneously from the epigastric angle under echocardiographic guidance. A total of 150 μCi of FIAU, labeled with 125 iodine as previously described, was injected intravenously 3 days later. Animals were killed after 24 hours. Hearts were rapidly excised, rinsed with saline, and frozen. Vertical long-axis slices with a thickness of 20 μm were prepared (HM500OM microtome, Microm), and digital autoradiography (Phosphorimager 445SI, Molecular Dynamics) was performed.

### Results

#### FIAU Accumulation and Adenoviral Vector Concentration

Uptake rates for radiolabeled nucleosides at various MOIs are summarized in the Table. After AdHCMV-TK infection, FIAU uptake in H9c2 cells was comparable to that in DU145 cells, and it increased with increasing MOIs, supporting the susceptibility of cardiac cells to AdHCMV-TK. The largest increase of FIAU uptake was found between MOI 0 and MOI 10. Further increase was not a linear function. Instead, a plateau was suggested for higher MOIs. No significant FIAU uptake was observed for negative controls, supporting the specificity of FIAU for HSV1-tk.

### Time Course of FIAU Accumulation After HSV1-tk Transduction

The time course of FIAU accumulation after infection, corrected for cell proliferation as measured by TdR uptake, is shown in Figure 2. In controls, uptake increased rapidly between 24 and 72 hours and reached a plateau between 96 and 120 hours. In H9c2 cardiac cells, the rapid increase occurred later (between 72 and 120 hours), whereas uptake in both cell lines was comparable at the latest time of 120 hours.

Negative controls revealed low TdR-corrected FIAU accumulation for DU145 cells (0.09 at 48 hours, 0.06 at 96 hours) and slightly

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**Tracer Uptake at Various Vector Concentrations**

<table>
<thead>
<tr>
<th>Vector (AdHCMV-TK)</th>
<th>MOI 0</th>
<th>MOI 10</th>
<th>MOI 25</th>
<th>MOI 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>H9c2 (HSV1-tk+)</td>
<td>FIAU (% appl dose) 0.04</td>
<td>2.92</td>
<td>5.30</td>
<td>6.36</td>
</tr>
<tr>
<td></td>
<td>TdR (% appl dose) 2.02</td>
<td>3.12</td>
<td>3.15</td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td>FIAU/TdR 0.02</td>
<td>0.93</td>
<td>1.69</td>
<td>1.94</td>
</tr>
<tr>
<td>H9c2 (control)</td>
<td>FIAU (% appl dose) 0.03</td>
<td>0.03</td>
<td>...</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>TdR (% appl dose) 2.22</td>
<td>2.10</td>
<td>...</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>FIAU/TdR 0.02</td>
<td>0.02</td>
<td>...</td>
<td>0.02</td>
</tr>
<tr>
<td>DU145 (HSV1-tk+)</td>
<td>FIAU (% appl dose) 0.19</td>
<td>5.60</td>
<td>5.00</td>
<td>5.07</td>
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<tr>
<td></td>
<td>TdR (% appl dose) 2.82</td>
<td>4.55</td>
<td>3.93</td>
<td>7.90</td>
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<tr>
<td></td>
<td>FIAU/TdR 0.07</td>
<td>1.23</td>
<td>1.27</td>
<td>1.56</td>
</tr>
<tr>
<td>DU145 (control)</td>
<td>FIAU (% appl dose) 0.22</td>
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<td>...</td>
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<tr>
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<td>TdR (% appl dose) 2.80</td>
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</tr>
<tr>
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<td>FIAU/TdR 0.08</td>
<td>0.09</td>
<td>0.12</td>
<td>...</td>
</tr>
</tbody>
</table>

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**Figure 1.** Imaging of cardiac gene expression using HSV1-tk and radiolabeled FIAU. FIAU is phosphorylated and trapped intracellularly only in presence of herpesvirus thymidine kinase.

**Figure 2.** Time course of [^14]C]-FIAU uptake in vitro after HSV1-tk transduction.
higher values for H9c2 (0.14 at 48 hours, 0.4 at 96 hours). These values never exceeded 15% of those of positive cells.

**In Vivo Results**

After the administration of AdHCMV-TK, the focally increased uptake of $^{125}$I-FIAU was clearly identified in the inferior wall, suggesting local expression of HSV1-tk after adenoviral gene transfer (Figure 3). An analysis of regional count rates revealed a 3.4±2.2-fold increase of $^{125}$I-activity in the area of vector administration compared with remote myocardium. An injection of the control vector did not result in significant tracer accumulation. Nonspecific uptake was <10% of positives (0.3±0.05-fold increase).

**Discussion**

The results demonstrate that the herpesvirus thymidine kinase gene can be used as a marker gene and that radiolabeled FIAU can be used as a marker substrate for the imaging of successful gene transfer and expression in cardiac tissue. After adenoviral transfer of HSV1-tk, FIAU uptake in cardiac cells in vitro was comparable to that of tumor cells serving as a positive control. Additionally, autoradiography clearly visualized the site of HSV1-tk expression after regional myocardial gene transfer in vivo. Thus, the imaging of transgene expression in the heart is feasible and may be used for the noninvasive monitoring of gene therapy.

Previously, the usefulness of HSV1-tk and radiolabeled FIAU has been demonstrated in tumors. High FIAU uptake was reported for retroviral transduced HSV1-tk–positive tumor cells in vitro and in vivo. However, gene expression was visualized with high target/nontarget ratios using conventional scintigraphy and positron emission tomography. In contrast to tumor cells, however, cardiac tissue has different cellular and molecular characteristics. In the present study, a molecular gene-targeted imaging approach was applied for the first time to myocardial cells. Our results support the feasibility of monitoring gene therapy not only in tumors, but also in cardiac tissue. In vitro results in proliferating cardiomyoblasts were transferable to adult cardiomyocytes in the in vivo setting. Further studies, however, will be needed to establish a quantitative relationship between FIAU uptake and transgene expression in vivo.

The combination of adenoviral vector, HSV1-tk, and radiolabeled FIAU was chosen as a model to visualize successful cardiac gene transfer and consecutive gene expression. Some limitations of this model, however, need to be recognized.

Although the high transduction efficiency of adenoviral vectors is supported by higher in vitro FIAU uptake after HSV1-tk transduction in this study compared with retrovirally transduced tumor cells in previous studies, the rapid elimination of HSV1-tk due to immunologic reactions against the vector, in addition to reactions against the transgene, may be a potential drawback in human applications. The concept of marker gene/substrate, however, is vector-independent. It can also be used to monitor gene transfer that is based on nonviral or less immunogenic viral vectors, such as adenovirus/associated viruses. For any type of therapeutic cardiac gene transfer, effector genes may be coexpressed with marker genes to determine the localization, extent, and magnitude of gene expression via the imaging of marker substrate accumulation.

Furthermore, it must be recognized that HSV1-tk not only represents a suitable marker gene, but can also act as a therapeutic “suicide gene” when combined with antiviral drugs such as ganciclovir in therapeutic doses. Therefore, precautions will be necessary when using HSV1-tk for cardiac gene imaging in the in vivo setting, because antiviral therapy may result in damage to transduced cardiomyocytes. FIAU itself has also been used as antiviral agent, but diagnostic doses of radiolabeled FIAU contain very small amounts (pmol) of the pharmaceutical and are therefore considered safe and nontoxic.

Finally, HSV1-tk and radiolabeled FIAU are only one among several potentially suitable combinations of marker genes and substrates for imaging. In the future, other combinations may be evaluated. This will contribute to the development of a clinically applicable approach for the noninvasive imaging of cardiac transgene expression.

**References**

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