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Reporter Gene Imaging Following Percutaneous Delivery in Swine:

Moving Toward Clinical Applications

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To the Editor

Noninvasive monitoring of cardiac gene therapy is critical to fully understand the biology of gene therapy in living subjects. We and others have monitored reporter gene expression in the myocardium of small (1) and large (2) animals (reviewed in reference 3). However, before these strategies are translated to the clinic, it is critical that they be tested using minimally invasive gene delivery approaches similar to those used clinically.

We tested the hypothesis that reporter genes can be delivered using a minimally invasive strategy to the myocardium of a swine, and expression can then be imaged using combined positron emission tomography-computed tomography (PET-CT).

Stanford's Animal Care and Use Committee approved all procedures. Six domestic pigs (Pork Power Farms, Turlock, California) were used in the study. With sterile technique, 8-F vascular sheaths placed in the carotid arteries were used for vascular access. Percutaneous endomyocardial delivery systems (Biocardia, Inc., South San Francisco, California) (Fig. 1, left panel), placed into the sheaths, were used for fluoroscopy-guided delivery of genes (10^{10} plaque-forming units) or vehicle (phosphate-buffered saline [PBS]) (Fig. 1, right panel) in 3 doses of 0.2 cc each. Central mean arterial pressure (MAP) was measured. Forty-eight hours after gene delivery, animals were dynamically imaged after intravenous administration of ^{18}F-labeled 9-[4-fluoro-3-(hydroxymethyl)butyl]guanine (^{18}F-FHBG; tracer) (4) using a clinical combined PET-CT system (Discovery LS, GE Medical Systems, Milwaukee, Wisconsin) for a total scanning time of 180 min. Data are expressed as mean ± SEM.

There were no significant differences in weight (control, 37.4 ± 0.4 kg; gene therapy, 36.3 ± 0.7 kg; p = NS), MAP (control, 107 ± 11 mm Hg; gene therapy, 111 ± 14 mm Hg; p = NS), or heart rate (control, 90 ± 7 beats/min; gene therapy, 95 ± 9 beats/min; p = NS) between the 2 groups. There was no morbidity or mortality associated with the procedures. A total of 9.37 ± 1.31 mCi of ^{18}F-FHBG (in 5 ml of PBS) was administered per animal.

Figure 2 (top panel, A to D) shows a representative PET-CT scan of the gene therapy group. The CT images (Fig. 2A, top panel) were used for anatomic localization, and ^{18}F-FHBG uptake (Fig. 2B, top panel) was located in the area into which gene therapy was delivered (anteroseptum) (Fig. 2C, top panel). Whole-body images (Fig. 2D, top panel) clearly showed the cardiac uptake in chest and abdominal structures.

Animals from both groups had comparable ^{18}F-FHBG uptake in paraspinal muscles and the nondelivered myocardial wall (Figs. 2E and 2F). Whereas control animals showed no distinct myocardial tracer uptake, experimental animals had significantly increased ($p < 0.05$) ^{18}F-FHBG uptake (Figs. 2E and 2F, respectively). The best myocardial signal/background (left ventricular) ratio was obtained 3 h post-injection (180 min, 4.63 ± 1.4 vs. 90 min, 1.78 ± 0.6; $p < 0.05$). Autoradiography and microPET confirmed the increased ^{18}F-FHBG uptake in the anteroseptum of the gene therapy animals.

Many different delivery methods have been developed for percutaneous cardiac delivery of gene therapy. The helical needle injection catheter system, used in this study, has the theoretic advantages of endocardial engagement and helical needle-track and has been shown to have good acute delivery success and retention (5). This delivery method has been designed to deliver material (e.g., genes, cells) to a specific and delimited area. Multiple injections or vascular-based delivery methods (e.g., intracoronary) may be more useful if the target area is a larger myocardial region or a specific coronary distribution.

Adenoviral infection results in strong, albeit relatively short-lived, transgene expression (6). For performing long-term longitudinal monitoring of therapy, other reporter gene strategies will be needed, such as adeno-associated or gutless adenovirus (7,8).

PET has nanomolar to picomolar (10^{-12} mol/l) sensitivity and tomographic capabilities, which makes PET the most suitable imaging modality for use in living subjects (compared with magnetic resonance and single photon emission-computed tomography) (9). Based on this study, 3 h after tracer administration appears to be a good time point for assessment of ^{18}F-FHBG uptake in the myocardium.

These studies will play a critical role in the monitoring of gene therapy first in pre-clinical large animal models of cardiac disease and then in clinical therapeutic trials.

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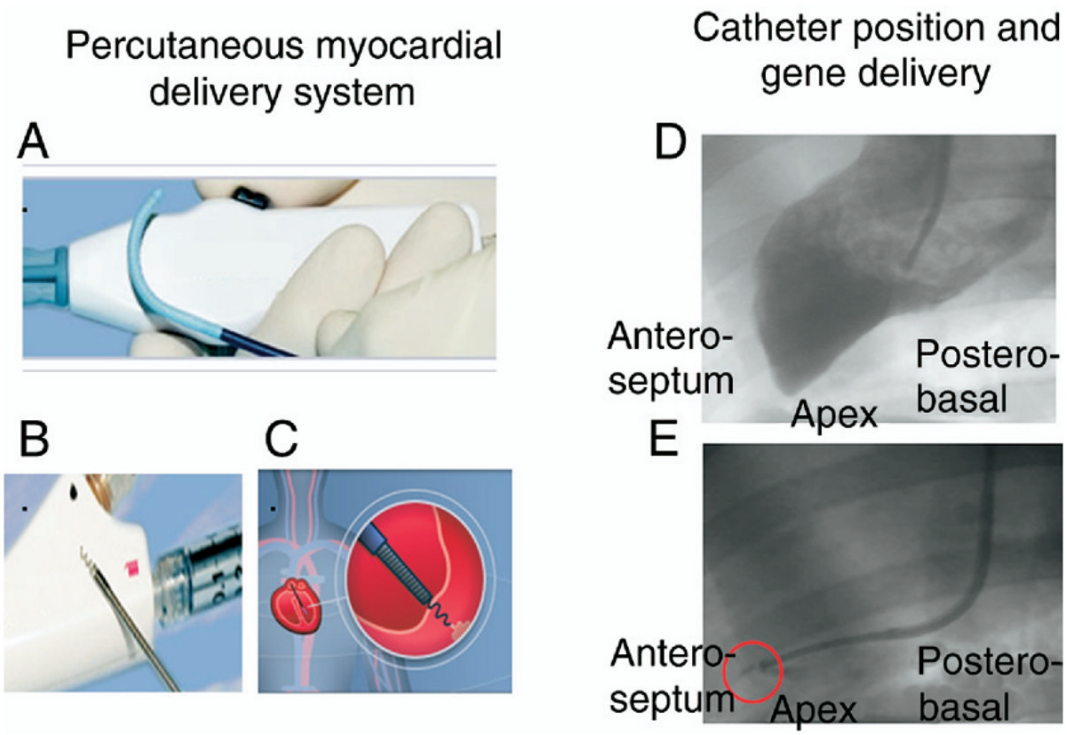

Figure 1. Fluoroscopy-Guided Delivery of Genes

(Left) Percutaneous delivery system consisting of **(A)** steerable-guiding catheter and **(B)** helical needle infusion catheter. A steerable catheter provides maximum flexibility, allowing catheter positioning in virtually any area of the left ventricular cavity. The infusion catheter is used first to confirm intramyocardial positioning of the catheter and then for the delivery of therapeutic material. **(C)** The infusion catheter is "screwed' inside the myocardium. **(Right)** Representative image of gene delivery in a swine model. A left ventricular angiogram is performed for delineation of the left ventricular endocardial contour **(D)**. Intramyocardial positioning of the helical catheter is confirmed using contrast media **(E)**, and gene therapy is then delivered.

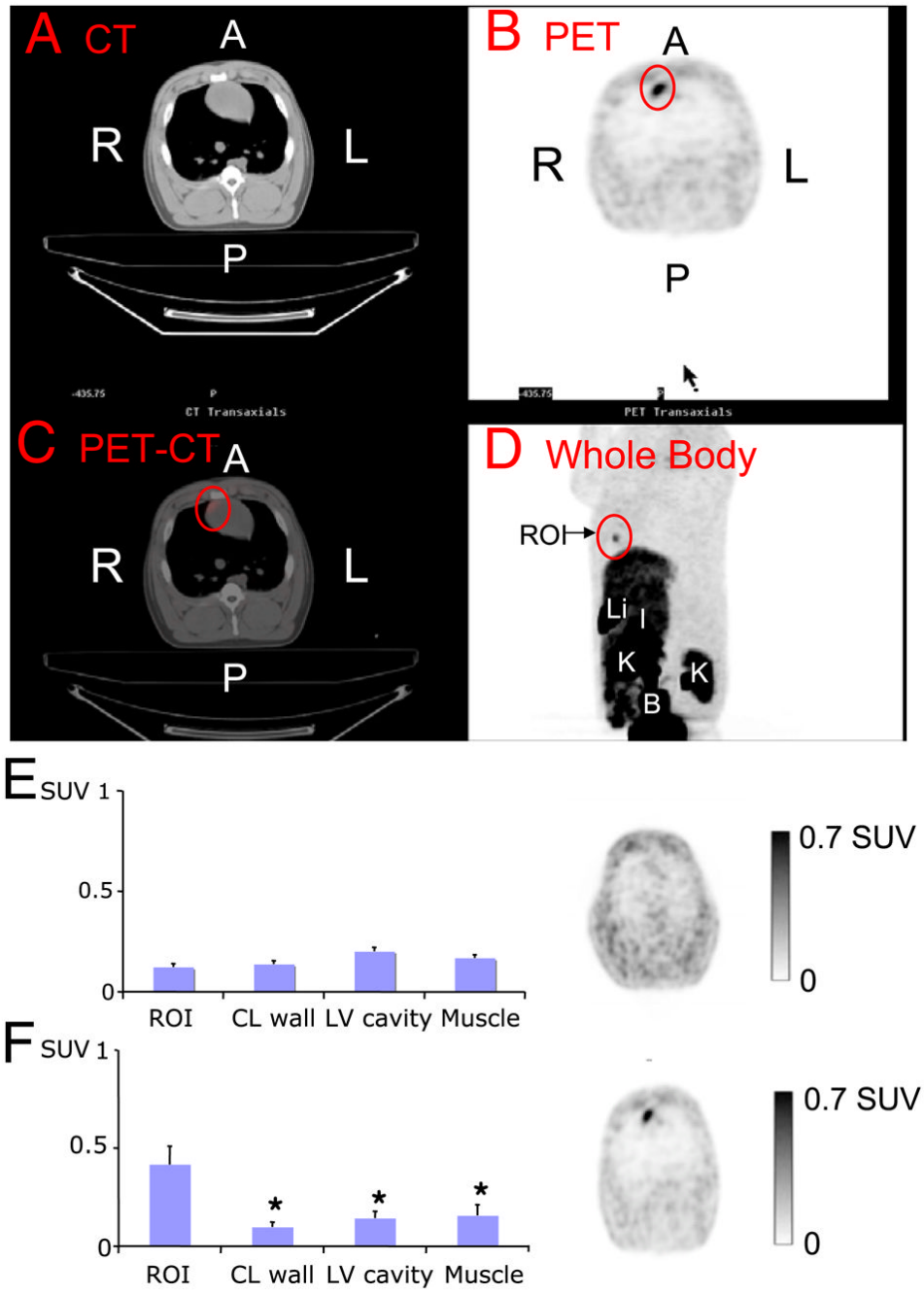

Figure 2. Representative Positron Emission Tomography (PET) Computed Tomography (CT)

(Top) Image of gene therapy 48 h after percutaneous gene delivery **(A to D). (Bottom)** Uptake of ^{18}F-labeled 9-[4-fluoro-3-(hydroxymethyl)butyl]guanine (standardized uptake value [SUV]) in **(E)** control (n = 2) and **(F)** gene therapy (n = 4) groups. *$p < 0.05$ compared to region of interest (ROI) **(red circle)**. A = anterior; B = bladder; CL = contralateral; I = intestines; K = kidney; L = left; Li = liver; LV = left ventricular; P = posterior; R = right.