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 (10¹⁰ 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 ¹⁸F-labeled 9-[4-fluoro-3-(hydroxymethyl)butyl]guanine (¹⁸F-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 ¹⁸F-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 ¹⁸F-FHBG uptake (Fig. 2B, top panel) was located in the area into which gene

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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.
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 18F-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 uptake in the anteroseptum of the gene therapy (Fig. 2C, top panel). Whole-body PET images obtained 3 h post-injection (180 min, 4.63 ± 0.6; p < 0.05) were used to compare different injection sites. Autoradiography and microPET confirmed the increased 18F-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 adeno virus (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 18F-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.

Martin Rodriguez-Porcel, MD
Todd J. Brinton, MD
Ian Y. Chen, MSE
Olivier Gheyens, MD
Jennifer Lyons
Fumiaki Ikeno, MD
Jurgen K. Willmann, MD
Lily Wu, MD, PhD
Joseph C. Wu, MD, PhD
Alan C. Yeung, MD
Paul Yock, MD
*Sanjiv Sam Gambhir, MD, PhD

*Stanford University School of Medicine
Bio-X Program, Departments of Radiology and Bioengineering
The James H. Clark Center
318 Campus Drive, Clark E150
Stanford, California 94305-5427
E-mail: sgambhir@stanford.edu

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REFERENCES

I read with great interest the paper by Cortigiani et al. (1). This intriguing study confirmed that coronary flow reserve (CFR) provides independent prognostic information in diabetic and nondiabetic patients with known or suspected coronary artery disease (CAD) and negative dipyridamole stress echocardiography. I feel that a few additional comments are necessary.

It is well known that aortic distensibility and CFR as characteristics of coronary microcirculatory function are reduced in diabetes mellitus (DM) (2). Moreover, aortic stiffening may lead to early pulse wave reflection, causing an increase in central systolic blood pressure (BP), a decrease in diastolic BP, and an increase in pulse pressure. The elevation in systolic BP increases myocardial oxygen demand, reduces left ventricular ejection fraction, increases ventricular overload, and induces left ventricular hypertrophy. Because myocardial blood supply depends largely on pressure throughout diastole and the duration of diastole, the contemporary decrease in diastolic BP can compromise coronary perfusion, resulting in subendocardial ischemia (3). Reduction in CFR was found in patients with increased aortic stiffness compared with age-, gender-, and risk factor–matched controls with normal aortic distensibility (4). These findings direct our attention to consider aortic stiffness as an important parameter affecting coronary hemodynamics.

The prognostic role of CFR and DM in patients with known or suspected CAD has been confirmed, demonstrating that both variables are independently predictive of cardiovascular survival (5). In recent studies, it has been demonstrated that CFR and indices describing aortic distensibility can be measured simultaneously by echocardiography, helping us better understand their relationship to each other (4,6). To see whether aortic distensibility could add predictive value, patients with and without CAD were examined (7,8). It was found in patients with CAD that aortic distensibility did not offer any added information in predicting cardiovascular survival. The potential complementary prognostic value of aortic distensibility for prediction of cardiovascular mortality over CFR was found in patients without CAD and abnormal CFR, which should be the topic of future research (8).

Interestingly, the number of studies evaluating the relationship between CFR and aortic stiffness in DM is limited (2,9,10). Alterations were found in CFR and aortic distensibility indices with correlations in diabetic patients with normal epicardial coronary arteries (2). In diabetic versus nondiabetic patients with CAD, aortic distensibility was reduced, but CFR was similar (9). Moreover, patients with aortic valve stenosis and type 2 DM had similar CFR and aortic distensibility indices compared with nondiabetic patients with aortic valve stenosis (10).

It should be considered that theoretically the echocardiographic evaluation of aortic distensibility simultaneously with CFR measurement is a relatively easy and patient–friendly method. Further investigations are warranted to examine the direct effect of aortic stiffness on coronary perfusion, especially in patients with DM. Furthermore, studies should evaluate the effect of antidiabetic drugs on coronary perfusion and aortic elasticity as well. Finally, studies evaluating the prognostic role of a combination of indices characterizing aortic distensibility and CFR in DM are warranted.

Attila Nemes, MD, PhD, FESC

*Second Department of Medicine and Cardiology Center
Medical Faculty
University of Szeged
P.O. Box 427
H-6720 Szeged, Korányi fasor 6
Hungary
E-mail: nemes@in2nd.szote.u-szeged.hu

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