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Magnetic Resonance Imaging Permits In Vivo Monitoring of Catheter-Based Vascular Gene Delivery

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Background—Gene therapy is an exciting frontier in modern medicine. To date, most investigations about the imaging of gene therapy have primarily focused on noncardiovascular systems, and no in vivo imaging modalities are currently available for monitoring vascular gene therapy. The purpose of this study was to develop an in vivo imaging tool to monitor a catheter-based vascular gene delivery procedure.

Methods and Results—We produced gadolinium/blue dye and gadolinium/gene-vector media by mixing Magnevist with a trypan-blue or a lentiviral vector carrying a green fluorescent protein (GFP) gene. The gadolinium was used as an imaging marker for magnetic resonance (MR) imaging to visualize vessel wall enhancement, and the blue dye/GFP was used as a tissue stain marker for histology/immunohistochemistry to confirm the success of the transfer. Using Remedy gene delivery catheters, we transferred the gadolinium/blue dye (n=8) or gadolinium/GFP lentivirus (n=4) into the arteries of 12 pigs, that were monitored under high-resolution MR imaging. The results showed, in all 12 pigs, the gadolinium enhancement of the target vessel walls on MR imaging and the blue/GFP staining of the target vessel tissues with histology/immunohistochemistry. This study shows the potential of using MR imaging to dynamically visualize (1) where the gadolinium/genes are delivered; (2) how the target portion is marked; and (3) whether the gene transfer procedure causes complications.

Conclusions—We present a technical development that uses high-resolution MR imaging as an in vivo imaging tool to monitor catheter-based vascular gene delivery. (*Circulation*. 2001;104:1588-1590.)

Key Words: cardiovascular diseases ■ gene therapy ■ magnetic resonance imaging

Gene therapy is a rapidly expanding field with great potential for the treatment of cardiovascular diseases. Many genes have been shown to be useful for preventing acute thrombosis, blocking post-angioplasty restenosis, and stimulating the growth of new blood vessels (angiogenesis).^{1,2} Different gene delivery techniques for the vasculature have been developed, including (1) ex vivo gene delivery, (2) surgery-based delivery, (3) percutaneous delivery, and (4) catheter-based delivery.³ Of these techniques, the catheter-based approach seems to hold the most promise for vascular applications.⁴

Gene transfer with delivery catheters is currently performed under x-ray fluoroscopy, which displays, using a contrast medium, only the lumen of the vessel without providing direct imaging information about the vessel wall or atherosclerotic plaques. Therefore, one cannot properly monitor either the interaction between the genes and the atherosclerotic lesion or the existence and distribution of the genes within the target lesion during and after vascular gene transfer. Magnetic resonance (MR) technology offers great potential to fill this gap.

Cardiovascular MR imaging has some prominent advantages, including the ability to image the vessel wall, the

ability to make multiple diagnostic evaluations of organ function and morphology, and the ability to provide multiple image planes with no risk of ionizing radiation. In the present article, we present our recent technical development using MR imaging to monitor a catheter-based primary vascular gene delivery procedure in vivo.

Methods

Study Design

The present study included 2 sections. First, to establish the experimental protocol, we produced a gadolinium/blue dye medium by mixing Magnevist (an MR imaging agent) with trypan-blue medium. Second, to preclinically validate the technical development, we produced a gadolinium/gene-vector medium by mixing Magnevist with a lentiviral vector carrying a green fluorescent protein (GFP) gene. In the media, the gadolinium was used as an imaging marker for MR imaging to visualize the enhancement of the target vessel wall, whereas the blue dye/GFP was used as a tissue/biology stain marker for histology/immunohistochemistry to confirm the success of the gadolinium/blue dye/GFP lentivirus-mediated transfer. Using 12 Remedy gene delivery catheters (SCIMED), we delivered the gadolinium/blue dye (n=8) or gadolinium/GFP lentivirus (n=4) into the vessel walls of the left iliac or femoral arteries of 12 pigs under high resolution MR imaging. The contralateral vessels of the target arteries were not transferred to serve as controls.

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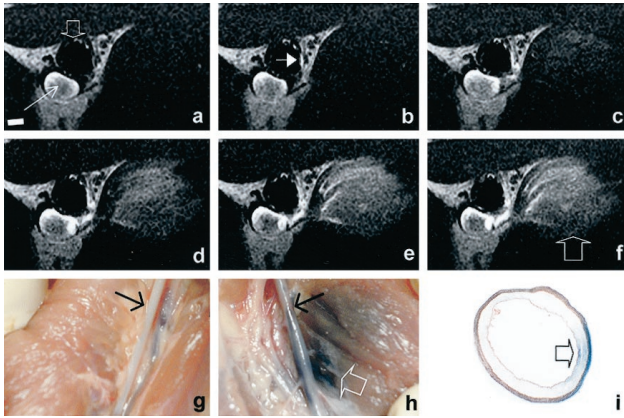


Figure 1. High-resolution MR images of the gadolinium/trypan blue delivery in the femoral artery of a pig. a, Before gadolinium/blue dye infusion. The open arrow indicates the artery, and the long arrow indicates the vein. Scale=1 mm. b through f, During gadolinium/blue dye infusion from minute 3 to minute 15 (at 3-minute intervals), the arterial wall is only partially enhanced by the gadolinium at 2 to 4 o'clock position (arrow in b). Gadolinium flows into the adjacent tissue (arrow in f) outside the target artery. g through i, Corresponding surgery and histology of both control and blue dye-targeted arteries. h, The blue dye stains the muscles (open arrow) outside the target artery (block arrow). There is no such finding in the control artery (g). i, Histology confirms that the blue dye primarily stains a portion of the artery wall at 2 to 4 o'clock with the wall dissection (arrow). Original magnification, 20 \times .

Gadolinium/Gene-Vector Medium

We constructed a novel lentiviral vector (EF.GFP) containing the human elongation factor (EF)-1 α promoter and the GFP gene (Y. Cui, PhD, unpublished data, 2001). Then, we mixed Magnevist with the GFP lentiviral vector to achieve a gadolinium/GFP lentivirus medium with a net Magnevist concentration of 6%. We previously confirmed that 3% to 6% of Magnevist (14 to 28 mg/mL gadolinium) is an optimum concentration for the MR imaging of balloon inflation.⁵

Gene Delivery Catheter Positioning

The Remedy gene delivery catheter has the dual capability of high-pressure lesion dilation and low-pressure gene infusion. It was positioned, using an 0.014-inch conventional guidewire, into the target arterial segment under x-ray fluoroscopy. Subsequently, we replaced the conventional guidewire with an 0.014-inch intravascular MR imaging-guidewire (Surgi-Vision).

MR Imaging

All experiments were performed on a 1.5 Tesla MR unit (GE). To image deeply located iliac arteries, we operated the MR imaging guidewire in a receive-only mode, but to image the superficially located femoral arteries, we used a custom-made, 3-cm surface coil. We first inflated the dilation balloon with 3% Magnevist and obtained a coronal scout MR image of the pelvis using a fast spoiled-gradient pulse sequence with a 500/2.1 ms repetition time/echo time, 31.2-kHz bandwidth, 24 \times 24-cm field of view, 256 \times 256 matrix, and 3 mm thickness. We then acquired an axial high-resolution MR image of the target arterial wall across the inflated balloon using (1) a spin-echo sequence (with 150/10 ms repetition/echo time, 16-kHz bandwidth, 6 \times 6 cm field of view, 128 \times 256 matrix, 1 to 3 numbers of excitation, and 3 mm thickness) and (2) a fast spoiled-gradient sequence (with 14.8/4.9 ms repetition/echo time, 15.6-kHz bandwidth, 4 \times 4 field of view, 256 \times 256 matrix, 8 numbers of excitation, and 3 mm thickness). The total scan time for each image was 1 minute. During the infusion of the gadolinium/blue dye/GFP lentivirus medium, the dilation balloon was inflated at 4 atm support pressure, and the medium infusion was constantly maintained using a pump (Harvard).

Histological and Immunohistochemical Confirmation

In the pigs infused with gadolinium/blue dye, we immediately harvested both target and control vessels for histopathological examination to confirm the success of the primary transfer. The pigs infused with gadolinium/GFP lentivirus were kept alive for 5 days to allow sufficient GFP expression. Then, we harvested both target and control arteries for immunohistochemical confirmation using a specific monoclonal antibody for GFP (Roche).⁶

Image Analysis

A region of interest was placed on the chosen portion of the target vessel wall to measure MR signal intensities. Then, we converted the measurements to obtain a signal intensity versus time curve. The results of MR images and histology/immunohistochemistry between the control and target tissues were analyzed blindly and were correlated by 3 investigators.

Results

In 2 of the 8 pigs used for the pilot studies, we initially infused the gadolinium/blue dye medium at a 5 mL/hour flow for 5 minutes under MR imaging. With these infusion parameters, we detected only mild and partial enhancement of the target vessel walls, which was confirmed by histology with several blue dye spots in the intima and its nearby media. We then increased the infusion flow to 10 mL/hour for 10 to 15 minutes in 4 pigs. After this infusion, we could dynamically visualize gadolinium enhancement within the entire vessel wall. The corresponding histology showed the blue dye stain in all layers of the intima and media, as well as the adventitia.

Subsequently, in the remaining 2 pigs, we tested the infusion of the gadolinium/blue dye medium at a higher flow of 20 mL/hour for 15 minutes. Under this condition, most of the gadolinium flowed into the areas outside the target vessels (Figure 1), which, on

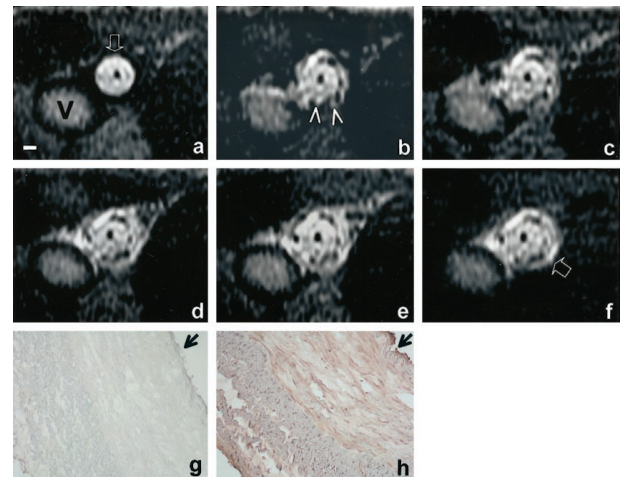


Figure 2. High-resolution MR images of the gadolinium/GFP lentivirus delivery in the iliac artery of a pig. a, Before gadolinium/GFP lentivirus infusion, the balloon is inflated with 3% Magnevist. The open arrow indicates the artery, and V, the vein. Scale=1 mm. b through f, During gadolinium/GFP lentivirus infusion from minute 3 to minute 15 (at 3-minute intervals), the arterial wall is enhanced by the gadolinium coming from the gene infusion channels (arrowheads in b) of the gene delivery catheter. At minute 15, the arterial wall is enhanced as a ring (arrow in f). g and h, Corresponding immunohistochemistry in both control (g) and GFP-targeted (h) arteries. h, GFP is detected as brown-colored precipitates through all layers of the intima (arrows) and media, as well as the adventitia. Original magnification, 200 \times .

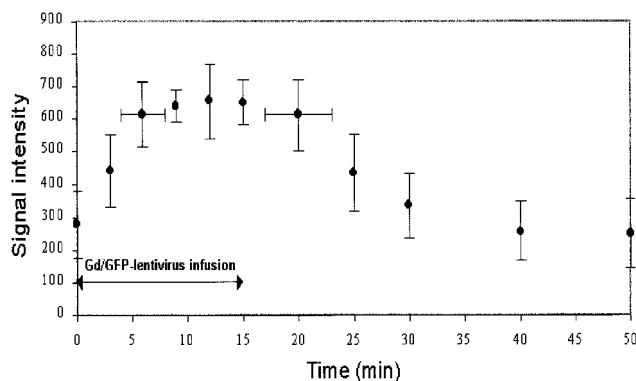


Figure 3. A curve of MR signal intensity of the target vessel wall versus the infusion time of the gadolinium (Gd)/GFP lentivirus delivery.

surgical examination, correlated with the blue dye stain in the muscles adjacent to the target vessels (Figure 1).

In the preclinical validation studies, the target vessel walls began to be enhanced immediately after initiating the gadolinium/GFP lentivirus infusion (Figure 2). The average time period for the target vessel walls to maintain peak signal intensity was from 6 ± 2 to minute 20 ± 3 minutes after the initiation of the gadolinium/GFP lentivirus infusion, and the signal intensity dropped to the preinfusion level within 40 to 50 minutes (Figure 3). After the gadolinium/gene transfer, all 4 pigs recovered. There were no clinical signs of ischemia in the treated extremities during the 5 days after the procedure.

The subsequent laboratory examination showed, in all 12 pigs, the blue dye/GFP staining of the target vessel tissues with histology/immunohistochemistry. The expression of GFP, as determined in situ by immunohistochemistry, indicated a functional in vivo gene transfer and expression with our approach. Immunohistochemistry showed that GFP was expressed through all layers of the target vessel wall (Figure 2).

Discussion

Monitoring catheter-based vascular gene delivery is critical. After the vascular gene transfer, clinicians need to assess the success of the primary gene therapy procedure immediately, including confirmation of where the genes are delivered and how the genes target the atherosclerotic lesions. Unrecognized failure of the primary vascular gene delivery can delay treatment for several months. However, proven failure of the primary gene delivery should indicate the need to replace the unsuccessful gene therapy with alternative treatment promptly. Currently, we are unable to assess the success of the primary gene delivery procedure because no in vivo imaging modalities are available for vascular gene therapy.⁷

Our study represents the first encouraging evidence that catheter-based vascular gene delivery can be monitored by

MR imaging in vivo. It shows the potential of using MR imaging to visualize dynamically (1) where the gadolinium/genes are delivered, (2) how satisfactorily the target portion of the vessel wall is marked, and (3) whether the gene transfer procedure causes complications, such as perforation, as shown in Figure 1. During catheter-based gene transfer in an atherosclerotic vessel, calcified and unevenly-thickened vessel wall can be easily perforated, which would need to be detected immediately. Our technical development should help clinicians to determine the reasons for a failed vascular gene transfer procedure and promptly decide on further management by either repeating the same gene therapeutic procedure or choosing an alternative treatment.

This study focused only on technical development using an easily prepared mixture of gadolinium contrast with gene vectors. Further work is required to use this new technique to quantify gene delivery/MR imaging protocols using different parameters (such as applied transmural pressure, viral solution volume and concentration, and gene delivery flow rate and infusion time) with various gene delivery catheters. With continuous improvement in the high-resolution imaging capability of MR technology, it will be possible to monitor details regarding the extent of gadolinium/gene transmural distribution. This technique shows the ability to confirm the success of the primary gadolinium/gene delivery but does not give sufficient insight into biological expressivity.

We present a technical development using high-resolution MR imaging as an in vivo imaging tool to monitor catheter-based primary vascular gene delivery. We think that this work opens up an exciting avenue for the future efficient management of cardiovascular ischemic disorders using MR imaging-based vascular gene/drug therapy.

Acknowledgments

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