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Intravascular Magnetic Resonance/Radiofrequency May Enhance Gene Therapy for Prevention of In-Stent Neointimal Hyperplasia

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Abstract

Rationale and Objectives—We evaluated the potential of using intravascular magnetic resonance (MR)/radiofrequency (RF) to enhance vascular endothelial growth factor (VEGF) gene therapy of in-stent neointimal hyperplasia.

Materials and Methods—Via a catheter-based approach, VEGF/lentivirus was locally transferred into 10 (five-paired) bilateral femoral-iliac arteries of five hypercholesterolemic pigs, while the right arteries were heated up to approximately 41°C using an intravascular MR/RF system. Then, identical stents were immediately placed into the bilateral VEGF-targeted arteries to create in-stent neointimal hyperplasia. At day 60 after gene/stent interventions, the targeted arteries were harvested for histology correlation.

Results—X-ray angiography-detectable in-stent stenoses were found in three of the arteries treated with VEGF genes only, while there were no in-stent stenoses in the arteries treated by MR/RF-heated VEGF genes. Correlative histology confirmed a 138% reduction of the average thickness of neointimal hyperplasia in VEGF/RF-treated arteries compared to VEGF-only-treated arteries ($p < 0.01$).

Conclusion—We report a potential method using an intravascular MR/RF-heating technique to enhance gene therapy of in-stent restenosis.

Keywords

atherosclerosis; cardiovascular diseases; gene therapy; stent placement; radiofrequency

Introduction

Atherosclerotic cardiovascular disease is the leading cause of death in developed countries (1). Along with balloon angioplasty, endovascular stent placement is one of the primary treatments for atherosclerotic cardiovascular disease, and significantly improves acute outcomes. However, in-stent restenosis is still a common clinical problem (2). Although drug-eluting stents offer potential for the treatment of atherosclerotic diseases, recent clinical trials involving drug-eluting stents have shown that the in-stent restenosis rate, so far, is still

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approximately 9% for all patients, 18% for diabetics, and 18% for patients with small vessel disease (3). The mechanisms of in-stent restenosis have not been fully elucidated; however, neointimal hyperplasia is considered one of the primary causes of in-stent restenosis (4).

Gene therapy provides great potential for reducing and inhibiting neointimal hyperplasia, and thus preventing in-stent restenosis (5). Several genes, such as vascular endothelial growth factor (VEGF), have been reported for use in preventing in-stent restenosis (6,7).

Due to current technological limitations, in vivo transfection/transduction of genes-vectors in the vasculature is low (5). This is one of the major hurdles of vascular gene therapy. Previous studies have demonstrated that controlled heating can enhance gene transfection/expression (8,9). This concept motivated the development of a technique using intravascular magnetic resonance (MR)-mediated radiofrequency (RF) to enhance vascular gene transfection and expression by designing an MR imaging-heating-guide wire (MRIHG) as an intravascular thermal energy source (10,11).

The purpose of the present study was to translate this new technique to a preclinical setting, to further evaluate the possibility of using intravascular MRIHG-mediated RF heat to enhance VEGF gene therapy for the prevention of in-stent neointimal hyperplasia.

Materials and Methods

Preparation of VEGF/lentivirus

VEGF has been documented to inhibit neointimal hyperplasia in vivo (6,7). Therefore, we chose to use this gene to demonstrate that MR-mediated RF heating would enhance expression of this gene in target vascular cells and, in doing so, effectively decrease the amount of neointimal hyperplasia associated with stent placement in a cholesterol-fed animal. Lentivirus-based gene therapy has been evaluated in Phase I Clinical Trials (*VIRxSYS-Corporation. Phase I clinical trial using VRX496 lentiviral vector. November 2004*). Thus, we used a lentivirus vector to carry a therapeutic gene, vascular endothelial growth factor (VEGF₁₆₅) gene (GeneCopoeia, Frederick, MD), and a marker gene, red fluorescent protein (RFP) gene. In the present study, the titers of viral supernatants were in the range of 1×10^6 to 6×10^6 transducing units (TUs) per milliliter of supernatants.

MR/RF-heating system

The primary portion of the MR/RF-heating system was an 0.014-inch (0.35-mm), copper-based intravascular MR imaging-heating-guidewire (MRIHG), which consisted of a coaxial cable, 5 feet (152.4-cm) in length with a 4.5-cm extension of the inner conductor (Microstock, Inc., West Point, PA). The MRIHG was connected to a custom external 180-MHz RF generator to deliver RF thermal energy to the target vessels.

Prior to applying it in the present study, the functionality of the MR-imaging/RF-heating system had been validated in vivo. By comparing different input powers, we established an optimized MR/RF heating protocol, which enabled us to achieve a temperature increase up to approximately 41°C from 37°C in the target femoral arteries of pigs by operating the 180-MHz RF generator at 4 watts through the MRIHG (Qiu B, et al. unpublished data).

Animals

This in vivo study was performed in 10 (five-paired) bilateral femoral-iliac arteries of five cholesterol-fed pigs, approximately 20 kg in weight (Archar, Baltimore, MD). All animals were treated according to the "Principles of Laboratory Animal Care" of the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" (NIH

Publication No. 80-23, revised 1985). The Animal Care and Use Committee at our institution approved the experimental protocol.

Gene delivery and stent placement

We used a gene delivery balloon catheter (Remedy; Scimed/Boston Scientific, Maple Grove, Minn) to locally deliver the VEGF/lentivirus into the target vessel wall using a previously-established gene delivery protocol (12). Through a surgical cutdown in the carotid artery, we placed a 7F introducer into the upper abdominal aorta. Heparin (Elkins-Sinn, Inc., Cherry Hill, NJ,) was intravenously administered (100IU/kg) to initiate anticoagulation. We then obtained a digital subtraction angiography (DSA) of the pelvic and bilateral femoral-iliac arteries in two perpendicular planes. Based on the results of the DSA, we selected the gene-targeted femoral-iliac artery segments that did not include branches. Then, via the gene delivery balloon, we delivered 1.0–2.0-mL VEGF/lentivirus into each of the bilateral arteries at a flow of 10mL/hour. The gene infusion was maintained using a digital syringe pump (Harvard, Holliston, MA). The amounts of transferred gene/vectors were exactly the same in both sides of each animal, and was precisely controlled by the same infusion time using the same infusion parameters set with the digital pump. The duration of the balloon inflation with gene infusion was 2 minutes for four times with a 2-minute interval to restore blood flow.

Before gene delivery, the 0.014-inch MRIHG was placed into the guidewire channel of the catheter and was connected to the external RF generator. From the beginning of gene delivery, we used the previously established MR/RF heating protocol to locally heat the unilateral gene-targeted artery segments up to approximately 41 °C for 20 minutes, while the contralateral gene-targeted arteries were not heated to serve as controls.

Subsequently, we placed 10 identical, stainless steel, balloon-expandable stents (BeStent™, Minneapolis, Minn) into the 10 VEGF-targeted bilateral femoral-iliac arteries. Figure 1 presents the experimental design and steps in the in vivo validation studies. All stents were primarily used as inductive devices to facilitate the formation of neointimal hyperplasia, i.e., in-stent stenosis. For each of the artery segments, the diameter ratios between the targeted artery and the stent were approximately 2.5mm/3.0mm, or 3.0mm/3.5mm. Immediately after the stent placement, we obtained DSA to confirm the success of the procedure.

After gene/stent interventions, we kept the pig alive, with a continuation of the high cholesterol diet (Modified Laboratory Mini-Pig Diet Grower 5081, TestDiet, Richmond, IN) for an additional two months. At day 60 after the gene/stent interventions, we obtained DSA again to examine the formation of in-stent stenosis in bilateral targeted arteries. Then, we euthanized the animals and harvested the bilateral, gene-targeted and/or stented artery segments for pathology correlation and confirmation. Since this study focused on a technical development, we evaluated only the short-term therapeutic effect of VEGF (two-months after gene/stent interventions) with no attempt to evaluate the long-term functional period of VEGF gene expression in the arterial tissues.

Pathology examination

All of the stent-containing artery specimens were fixed with 10% buffered formalin, embedded in methylmethacrylate, and sectioned at 5-μm-thick slides at three levels of the proximal and distal ends, as well as the middle portions of the stented artery segment using a laser microtome (Division of Charles River Laboratories, Inc., Frederick, MD). The histological slides were then stained with hematoxylin and eosin (HE).

Analysis

With microscopy, we photographed 30 histological slides (10 artery segments x 3 slides per segment = 30 slides), cross-sectionally viewed at 13.6-times magnification. On each of the 30 photographs, we recorded the average thickness of the in-stent neointimal hyperplasia by measuring the shortest distance from the inner margin of the stent to the endothelial layer of neointimal hyperplasia. Thus, a total of 249 measurements were recorded, including 123 measurements for VEGF+RF-arteries and 126 measurements for VEGF-only-arteries. Unequal numbers of two measurements were due to the variable number of stent structures that appeared in different histology slides/photographs. An unpaired Student's *t* test was used to compare the differences in the average thickness of in-stent neointimal hyperplasia in the arteries between the VEGF-RF and the VEGF-only treatments. The data were given as mean $\pm$ standard error and considered significantly different at the level of $p < 0.05$.

Results

Gene delivery and/or stenting procedures were primarily successful in all 10 arteries, and all animals survived the procedures with no complications. DSA that was obtained 60 days after gene/stent interventions showed in-stent stenosis in three of five VEGF-only arteries, while all five of the VEGF/RF-treated arteries remained patent (Figure 2). Microscopy examination demonstrated that the average neointimal hyperplasia was 138% thinner in VEGF-RF-arteries than in VEGF-arteries (VEGF-RF/VEGF = $318.9 \pm 136.2 \mu\text{m}$ / $440.1 \pm 212.6 \mu\text{m}$, $p < 0.01$). The lumens of the VEGF-only-treated arteries were narrowed by neointimal proliferation, with specific disruption of the internal elastic lamina, mural compression, and mural inflammation. Endothelium-lined fibrous neointima covered the stent structures.

Discussion

Endovascular stenting exerts its effect through purely mechanical means, providing lumen scaffolding that decreases recoil. However, in-stent restenosis is still a common problem after interventional therapies on atherosclerotic vessels, even with drug-eluting stents, and is particularly prominent in the small arteries, such as the coronary arteries (13). Some studies have shown that the VEGF gene can not only promote angiogenesis (5), but also inhibit in-stent restenosis via its properties as an angiogenic, cytoprotective, and endothelial repair factor (6,7). In addition, previous studies have shown that heat can enhance gene transfection (8,9).

In clinical practice, it would be desirable to have an image-guided method to locally heat the target vessel segments only. One way to address this is to develop an internal heating source that would be small enough to be easily placed into a local target via naturally existing anatomic channels, such as vessels. This motivated the development of the intravascular MR-imaging/RF-heating system for simultaneous monitoring and enhancing of vascular gene therapy (10). A previous study has demonstrated the feasibility of using intravascular MR/RF to enhance vascular gene expression (11), while in the present study, we attempted to further evaluate the possibility of using this new technique to enhance vascular gene therapy of in-stent neointimal hyperplasia.

With direct histology confirmation, the present study demonstrates that VEGF gene therapy of in-stent neointimal hyperplasia can be enhanced by locally-controlled heat in vivo and by using the MR/RF-heating system with the 0.014-inch MRIHG as an intravascular local heating source. The proposed mechanisms for this may include the fact that heating can enhance gene transfer to the targets by tissue fracturing, increase the permeability of the plasma membrane and cell metabolism, and increase the activity of heat-sensitive heat shock proteins (9,14,15).

The primary objective of the current study was to test the possibility of using the intravascular MR/RF-heating technique to enhance VEGF gene therapy of in-stent stenosis. Thus, to arrange comparative experiments by placing identical stents in the paired artery segments of each pig would be considered desirable and reasonable. The two-minute occlusion with the gene delivery balloon would probably not be practical in the coronary circulation. This problem could be solved by using a gene delivery balloon/infusion catheter with build-in infusion channels. The infusion channels would permit constant blood flow through the inflated balloon. Alternatively, sufficient blood flow through the occluded vessel segment could be achieved by shortening the balloon inflation time with increasing the numbers of balloon inflation/deflation cycles to permit adequate gene delivery.

Limitation of this study includes the small number of animals reported due to the availability of the same types of stents, and no perfusion-fixation of the vessels. Further work needs also to quantitatively validate this new technique using different heating parameters and various gene/vectors with controls.

In summary, this study demonstrates the potential of using an intravascular MR/RF-heating technique to enhance gene therapy for the prevention of in-stent neointimal hyperplasia.

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Figure 1.

Experimental design for MR/RF-enhanced VEGF gene therapy to prevent in-stent neointimal hyperplasia, including a) initiation of cholesterol diet; b) delivery of VEGF genes to bilateral femoral-iliac arteries with local MR/RF heat on the left side; c) placement of identical stents in gene-targeted bilateral artery segments; d) continuation of cholesterol diet for additional two months; e) formation of in-stent neointimal hyperplasia at the right femoral-iliac artery while the left corresponding artery is still patent due to RF-enhanced VEGF gene therapy; and f) harvesting targeted tissues for pathology correlation. MRIHG=magnetic resonance imaging-heating-guidewire.

Figure 2.

Comparison between VEGF-only treated and VEGF/RF-treated arteries. (A) X-ray angiography obtained two months after stenting shows the left femoral artery still patent (solid arrow) due to MR/RF-enhanced VEGF gene treatment, while the right femoral artery is narrowed (open arrow). Collaterals are also visualized at the right side (arrowheads). (B) A plot shows that the average thickness of neointimal hyperplasia in VEGF-treated arteries is higher than that in VEGF/RF-treated arteries for the five individual pigs. (C&D) Microscopy examination of the stented arteries with VEGF gene therapy (C) and with RF-enhanced VEGF gene therapy (D). Neointimal hyperplasia (N) is thinner in VEGF/RF-treated artery (D) than in VEGF-treated artery (C). S=stent structures, M=Media, L=lumen. H&E staining, 68X.