Intravascular MR/RF-Enhanced VEGF Gene Therapy of Atherosclerotic In-Stent Restenosis

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Introduction

Atherosclerotic cardiovascular disease remains the leading cause of death in developed countries. Along with balloon angioplasty, endovascular stent placement is one of the primary treatments for atherosclerotic cardiovascular disease. In-stent restenosis is a frequent clinical problem, occurring in 10% to 40% of patients who receive endovascular stenting with conventional stents. The mechanism of in-stent restenosis has not been fully elucidated, and neointimal hyperplasia is considered the primary reason for in-stent restenosis. Gene therapy provides great potential to reduce neointimal hyperplasia, and thusprevent in-stent or post-angioplasty restenosis (1). Several genes, such as vascular endothelial growth factor (VEGF) genes, have been approved for the treatment of restenosis (2, 3). However, currently, the in vivo transfection/transduction of genes-vectors in vasculatures is very low. To solve this problem, we have recently developed a new technique of intravascular MR/RF-enhanced vascular gene transduction/expression (4, 5). The purpose of the present study was to validate this new technique in a preclinical setting, using intravascluar MR-mediated RF heat to enhance VEGF gene therapy of atherosclerotic in-stent restenosis in pigs.

Materials and methods

We used 18 (9-paired) femoral/iliac arteries of nine domnestic pigs (11.6±2.2kg in weight). Cholesterolemia was first created by feeding the pigs an atherogenic diet (TestDiet, Indiana) for two months. The levels of circulated cholesterol and triglycerides were examined at day 0 (as baseline data) and at day 60 after stent placement. The pigs were divided to two groups. In the first group of four pigs, we confirmed initially the anti-in-stent restenosis effect of VEGF/lentivirus compared to that of GFP/lentivirus (as a negative control). We delivered 1-mL VEGF/lentivirus into the unilateral femoral/iliac artery and 1-mL GFP/lentivirus into the controlateral femoral/iliac artery (as a control). Then, the same-sized stents (BeStent™, MN) were placed in the bilateral gene-targeted femoral/iliac arteries of each pig. In the second group of five pigs, we compared the therapeutic effect of VEGF genes between MR/RF-heated and non-MR/RF-heated arteries of each pig. We first delivered 1 mL VEGF/lentivirus into bilateral femoral/iliac arteries. Then, we placed the same-sized stents into the VEGF-targeted bilateral femoral/iliac arteries, while the unilateral gene-targeted femoral/iliac artery was heated by providing intravascular MR-mediated RF thermal energy for 20 minutes and the controlateral gene-targeted femoral/iliac artery was not heated (control). The diameter selection of the gene delivery balloon catheters and the stents was based on a conventional angiogram, which outlined both the pelvic arteries and the femoral arteries on both sides. We selected a 2.5–3.5-mm-diameter, 20-mm-long portion of each of the bilateral femoral/iliac arteries as the gene/stent-targeted vessels. The diameter ratio between the targeted arterial portion and the gene delivery balloon/stent was 2.5-3.5/3.0-4.0, respectively.

At day 60 after gene delivery/stent placement, we harvested the bilateral targeted femoral/iliac artery segments for pathology correlation. The arterial specimens were embedded in methylmethacrylate, sectioned on a 5-µm-thick slide using a diamond-coated band saw (Exakt™ System), and surface-stained with hematoxylin & eosin (HE). Each of the 18 stent-containing artery segments was sectioned at five levels, including one slide at the proximal edge of the stent, three at the stent-containing segment, and one at distal edge of the stent. For statistical analysis, a total of 54 cross-sectionally viewed pathology slides were microscopically photographed at 13.6X magnification. We then recorded the thickness of in-stent neointimal hyperplasia by measuring the shortest distance from the inner margin of the stent to the luminal surface of the intimal layer of the neointimal hyperplasia. Data are given as mean±standard error. We used un-paired Student *t* tests to compare the difference in neointimal hyperplasia thickness between VEGF and GFP arteries, as well as between VEGF and VEGF+RF-heated arteries. Data were considered significantly different if $P < 0.05$ was obtained.

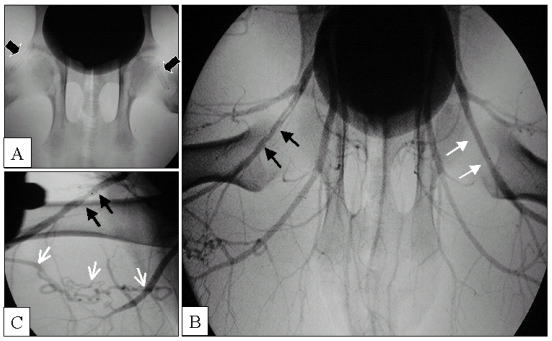

Fig. 1. (A) X-ray image shows two stents (arrows) placed in the bilateral femoral arteries of a pig. (B) X-ray angiogrpahy obtained 60 days after stent placement and gene therapy shows the atherosclerotic in-stent restenosis (black arrows) in the right GFP (control) gene-treated femoral artery, while the left corresponding stented femoral artery is still patent due to VEGF gene therapy. (C) X-ray angiography shows collaterals (white arrows) due to in-stent restenosis (black arrows) on the same side.

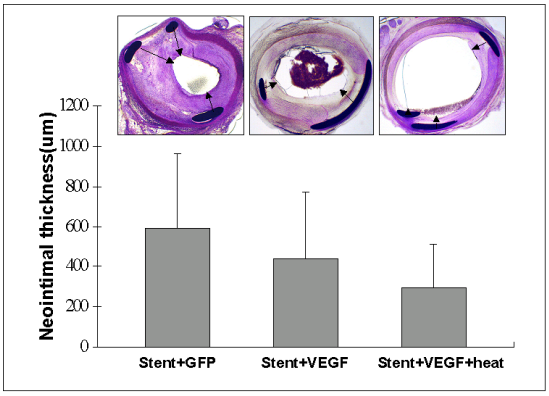

Fig. 2. Comparison of average neointimal thickness among different treatment groups of (left) stent+GFP gene; (middle) stent+VEGF gene; and (right) stent+VEGF+RF heating. The most severe neointimal hyperplasia was detected in the stent+GFP gene-treated arteries, less in stent+VEGF gene-treated arteries, and least in the stent+VEGF gene+RF heat-treated arteries. Arrows on the pathology slides demonstrate measurement of neointimal thickness.

Results

In all of nine pigs, cholesterol levels increased from 103.3±35.5mg/dL at baseline to 602.1± 319.5mg/dL at day 60 after gene/stent interventions. In the group comparing VEGF gene therapy with GFP gene therapy, all four angiographies obtained 60 days after gene transfer and stenting showed in-stent restenosis in the GFP-treated arteries, while no obvious restenosis was detected in the VEGF-treated arteries (Fig.1). In the group comparing MR/RF-heated VEGF gene therapy with non-heated VEGF gene therapy, x-ray angiography-detectable in-stent restenoses were found in three of five femoral/iliac arteries treated with VEGF genes only, while no in-stent restenoses were detected in the femoral arteries treated by VEGF genes plus RF heating. Microscopy examination of pathological slides presented clear differences in neointimal hyperplasia, which was thicker in GFP-treated arteries than in VEGF-treated arteries (Fig.2). Compared to the VEGF or VEGF-heated arteries, the GFP-treated arteries showed arterial lumens severely narrowed (>75%) by asymmetrical fibrous neointimal hyperplasia, with severe fragmentation of the internal elastic lamina, mural disruption, and marked chronic inflammation with large, foamy or vacuolated macrophages surrounding the stents (Fig.2). With regard to in-stent neointimal thickness, the average neointimal hyperplasia was thickest in GFP-treated arteries (590.51±369.41µm), less in VEGF-treated arteries (390.15±315.22µm, ($P<0.05$), and least in VEGF+RF-heated arteries (293.82±216.76µm, $P<0.05$) (Fig.2). Comparison of the in-stent restenosis between GFP-treated and VEGF plus RF-heated groups showed the most significant difference ($P<0.01$).

Conclusion

Our preliminary data shows that intravascular MR-mediated RF-heating offers great potential to enhance gene therapy of atherosclerotic in-stent restenosis.

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