Percutaneous coronary intervention is a major therapeutic option to treat cardiovascular disease and achieves a favorable outcome with low invasiveness in these patients. Introduction of bare metal stents has overcome elastic recoil and dissection at the site of intervention, and the advent of drug-eluting stents (DES) has effectively reduced in-stent restenosis. However, a DES cannot be implanted at all sites of lesion formation. Several clinical studies, including unselected patients, such as patients with complex coronary lesions or high-risk factors, revealed that ≤10% of patients with DES exhibited in-stent restenosis and required additional revascularization.1 In addition, DES have not proven to be well-suited to treat peripheral artery disease because of exposure to forces during joint flexion or external compression.2 DES have been also implicated in leading to increased risk of delayed restenosis and late thrombosis associated with impaired vascular healing.3 Although drug-coated balloon catheters have emerged as a potent therapeutic alternative in the interventional field, restenosis after angioplasty is still a major limitation and hampers the procedure's efficacy to treat cardiovascular disease.4

Background—Despite the advent of drug-eluting stents, restenosis after endovascular intervention is still a major limitation in the treatment of cardiovascular disease. To regulate the multiple biological mechanisms underlying restenosis, we focused on inhibition of an important transcription factor, nuclear factor-kappaB (NFκB), using a decoy strategy.

Methods and Results—For site-specific application of NFκB decoy oligodeoxynucleotides into target vessels during angioplasty, we developed a balloon catheter–based delivery system combined with biocompatible nanoparticles as oligodeoxynucleotides carriers. To clarify the therapeutic effect at the site of neointima, balloon angioplasty of the rabbit carotid arteries was performed at 4 weeks after initial endothelial denudation. This delivery system exhibited successful transfer of fluorescence-labeled nanospheres into the neointima in short-term contact with target vessels, and fluorescence could be detected ≥1 week after angioplasty. Consistently, local application of NFκB decoy oligodeoxynucleotides-loaded nanospheres resulted in significant inhibition of neointimal formation, associated with inhibition of NFκB binding activity in the injured arteries. The therapeutic effects were caused by inhibition of macrophage recruitment through the suppression of monocyte chemoattractant protein-1, vascular cell adhesion molecule-1, and CC chemokine ligand 4 expression and inhibition of vascular smooth muscle cell growth via a decrease in the expression of cyclin A and proliferating cell nuclear antigen. Importantly, application of NFκB nanospheres accelerated restoration of the endothelial cell monolayer, associated with enhanced expression of phosphorylated Bcl-2 in endothelial cells.

Conclusions—A drug-coated balloon catheter using NFκB decoy oligodeoxynucleotides significantly inhibited the development of neointimal hyperplasia in rabbits. The present study indicates the possibility of a novel therapeutic option to prevent restenosis after angioplasty. (Circ Cardiovasc Interv. 2014;7:787-796.)

Key Words: angioplasty ■ gene therapy ■ restenosis
WHAT IS KNOWN

- Decoy oligodeoxynucleotide–loaded poly(lactic-co-glycolic acid) nanospheres can be bound electrostatically to the balloon surface without additional agents.
- The poly(lactic-co-glycolic acid) nanosphere–coated angioplasty balloon is an effective approach to introduce decoy oligodeoxynucleotide to the vascular wall using short-term contact with the target vessels.

WHAT THE STUDY ADDS

- A drug-coated balloon catheter using nuclear factor-kappaB decoy oligodeoxynucleotide has the potential to treat stenotic lesions in which other endovascular techniques are not suited or are technically challenging.

homeostatic regulation of thrombosis and leukocyte adhesion. Infiltration of inflammatory cells and activation of vascular smooth muscle cells (VSMC) are also caused by vascular damage, resulting in enhanced secretion of various kinds of growth factors and cytokines. These responses facilitate VSMC growth and extracellular matrix deposition, leading to the progression of neointimal hyperplasia.\textsuperscript{5,6} Although several approaches have been proposed to regulate neointimal growth, modulation of only 1 molecule or pathway might have limited value against this multifactorial process. Therefore, we have focused on an important transcription factor, nuclear factor-kappaB (NFkB), because it is well known to play a critical role in the transcription of a variety of genes, primarily those related to inflammation.\textsuperscript{7} In addition, NFkB activity also appears to be essential for VSMC proliferation and migration and endothelial cell death.\textsuperscript{8,9} Importantly, several human studies identified activation of NFkB in vessels with atherosclerosis or after angioplasty, but not in normal arteries.\textsuperscript{10,11} Activated NFkB binds to the specific cis-element in the promoter region in the nucleus, leading to transactivation of a set of genes associated with physiological processes. To modulate endogenous transcriptional regulation, a decoy strategy has been used. Synthetic decoy oligodeoxynucleotides (ODN) contain the consensus sequence of the binding site of the target transcription factor. After delivery into the nucleus, decoy ODN can bind to free target transcription factors, resulting in blockade of the binding of these factors to the promoter regions.\textsuperscript{12,13} Our previous studies demonstrated that suppression of NFkB activity using a decoy strategy inhibited neointimal formation in several experimental models.\textsuperscript{14,15} However, there are some unresolved issues in the clinical application of ODN-based therapy, such as rapid degradation of ODN. In addition, although several balloon catheters for ODN transfer have been evaluated in animal models, these delivery systems have also limitations for clinical utility, including low transfection efficiency and vascular injury. To overcome these problems, we developed a decoy ODN-coated balloon (DCB) catheter using chitosan-modified poly(d,l-lactide-co-glycolide; PLGA) nanospheres (NS). The aim of this study was to clarify the efficacy of balloon angioplasty using the NFkB DCB catheter against neointimal formation in rabbit vascular injury models.

Figure 1. Release kinetics of NFkB-NS and characterization of NFkB DCB. A, Time course of dissolution of NFkB decoy oligodeoxynucleotides (ODN) from NFkB-NS. B, Electrostatic coating method on balloon surface. C, Uncoated balloon (left panel) and NFkB DCB (right panel). D, Field emission–type scanning electron microscope image of balloon surface of NFkB DCB. DCB indicates decoy-coated balloon; NFkB-NS, NFkB decoy ODN-loaded chitosan-modified PLGA nanospheres. Scale bar in D, 1000 nm.
Methods

Additional details are provided in the Data Supplement.

Synthesis of ODN and Selection of Target Sequences

The following sequences of double-stranded phosphorothioate decoy ODN were used:12,14

*NF*κB decoy ODN (consensus sequences are shown in italic):

5′-CCTTGAGGGATTTCCCTCC-3′

3′-GGAACTTCCCTAAAGGGAAGG-5′

Scrambled NF*κ*B decoy ODN:

5′-TTGCCGTACCTGACTTAGCC-3′

3′-AACGGCATGGACTGAATCGG-5′

Preparation of Chitosan-Modified PLGA NS Containing Decoy ODN

For administration of decoy ODN into target cells, we used a polymeric nanoparticle-based delivery system. Biocompatible PLGA NS were prepared by an emulsion solvent diffusion method, and the particle surface was modified with chitosan, as previously described.16,17 Using this preparation method, decoy ODN could be loaded on both the inner and outer layer of chitosan-modified PLGA NS. In this study, we used 3 types of NF*κ*B decoy ODN-loaded chitosan-modified PLGA NS (NF*κ*B-NS; Figure 1A): encapsulated type (ODN were entrapped in NS only), surface-supported type (ODN were bound to NS surface only), and encapsulated/surface-supported type (ODN were loaded both into and onto NS).

Preparation of Chitosan-Modified PLGA NS–Coated Balloon

Conventional angioplasty balloon catheters (length, 40 mm; diameter, 3.0 or 5.0 mm) were coated with chitosan-modified PLGA NS containing decoy ODN by an electrostatic coating method. The balloon surface was coated with a hydrophilic polymer, a methyl vinyl ether-maleic anhydride copolymer. This coating polymer was subjected to alkaline treatment to provide the balloon surface with anionic property. Decoy ODN-loaded chitosan-modified PLGA NS were dissolved in distilled water (10% wt/wt), and the balloon was immersed in this solution for 10 minutes and dried at low temperature. This procedure was repeated several times to coat a sufficient dose of PLGA NS on the balloon surface.

Table. Characterization of NF*κ*B-NS

<table>
<thead>
<tr>
<th>Type of NS</th>
<th>Mean Particle Size, nm</th>
<th>Zeta Potential, mV</th>
<th>Loading Efficiency of ODN, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified</td>
<td>280</td>
<td>−34.2</td>
<td>0</td>
</tr>
<tr>
<td>Encapsulated</td>
<td>360</td>
<td>+4.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Surface-supported</td>
<td>413</td>
<td>+55.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Encapsulated/surface-supported</td>
<td>533</td>
<td>+27.1</td>
<td>4.2</td>
</tr>
</tbody>
</table>

NF*κ*B indicates nuclear factor-kappaB; NS, nanosphere; ODN, oligodeoxynucleotides; and Unmodified, unmodified poly(d,l-lactide-co-glycolide) nanospheres with chitosan.

Figure 2. Prevention of neointimal formation in single vascular-injury model. A, Relative radioactivity of aorta after angioplasty with different inflation times of balloon (upper panel) and time course (lower panel). Radioactivity was determined as the ratio of concentration of radioactivity of the expanded abdominal aorta to that of the normal thoracic aorta (n=5 at each time point). B, Distribution of fluorescent isothiocyanate-labeled poly(d,l-lactide-co-glycolide) nanosphere. Typical photograph of fluorescence in aorta at 1 week after angioplasty. C, Representative histological sections of rabbit aorta stained with elastic van Gieson’s stain, and intimal, medial, and total areas in cross section and ratio of intimal to medial area at 4 weeks after angioplasty (n=5 per group). Sham, abdominal aorta from sham-operated rabbits; control, abdominal aorta treated with uncoated balloon; NF*κ*B (nuclear factor-kappaB), abdominal aorta treated with NF*κ*B decoy-coated balloon (DGB). *P <0.05 vs control. Scale bar in B, left panel, 500 μm; right panel, 100 μm; in C, 500 μm.
Experimental Animal Models and Angioplasty

Male Japanese white rabbits weighing 3.0 to 3.5 kg were used in this experiment. After systemic heparinization, an arterial embolotomy catheter (5F Fogarty balloon catheter) was introduced through the femoral sheath and advanced to the abdominal aorta under fluoroscopic guidance. To denude the endothelium, the balloon was inflated and withdrawn from the level of the right renal artery to the aortic bifurcation. Then, balloon angioplasty was performed using an NFκB DCB catheter or uncoated balloon catheter (length, 40 mm; diameter, 5 mm) in the infrarenal aorta (n=5 per group). The balloon was inflated at 10 atm for 1 minute with the balloon-to-artery ratio of 1:1.2. All animals did not receive antithrombotic drug after angioplasty.

To further investigate the efficacy of an NFκB DCB catheter in a clinically relevant situation, we used a modified double-injury rabbit model. After angiography, denudation of endothelium in the right common carotid arteries was performed using a 3F Fogarty balloon catheter. Four weeks after initial endothelial denudation, the occurrence of stenotic lesions was confirmed by angiography, and balloon angioplasty was performed in the right common carotid artery. The balloon (length, 40 mm; diameter, 3 mm) was inflated at 10 atm for 1 minute with the balloon-to-artery ratio of 1:1.2. Animals were divided into 4 groups: untreated (denudation of endothelium only, n=7), control (angioplasty with uncoated balloon, n=8), treatment with scrambled DCB (n=8), and treatment with NFκB DCB (n=11).

The experimental protocol was approved by the local Institutional Animal Care and Use Committee, and this study was performed under the supervision of the Animal Research Committee in accordance with the Guidelines on Animal Experiments of Osaka University Medical School and the Japanese Government Animal Protection and Management Law (No. 105).

Statistical Analysis

Statistical analysis was performed using JMP software package version 9.0 (SAS Institute Inc.). Normality of distribution was tested using the Shapiro–Wilk test. Continuous variables with normal distribution were expressed as mean±SEM, and unpaired t-test was used for comparison between 2 groups, and Tukey–Kramer multiple range test was used for comparisons among multiple groups. Variables with non-normal distribution were expressed as median and 25th to 75th percentiles, and nonparametric Kruskal–Wallis test was used for comparisons among multiple groups. P<0.05 was considered significant.

Results

Characterization of NFκB DCB Catheter

The mean particle size of NFκB-NS ranged from 280 to 533 nm. Although the zeta potential of unmodified PLGA NS showed a negative charge, that of NFκB-NS was shifted to a positive charge by modification with chitosan (Table). The dissolution study demonstrated that the surface-supported and encapsulated/surface-supported types released ≤90% of the initial amount of decoy ODN during 14 days. Interestingly, the encapsulated/surface-supported type of NFκB-NS showed sustained release of decoy ODN after the initial burst. This type released ≈25% of the loaded ODN in first 5 hours and ≈40% in 24 hours (Figure 1A). Based on these release profiles, we focused on the encapsulated/surface-supported type of NFκB-NS because its biphasic release profile was thought to be useful to prevent neointimal formation in both the acute and subacute responses after vascular injury. Therefore, the encapsulated/surface-supported type of scrambled decoy ODN-loaded chitosan-modified PLGA NS (scrambled-NS) and NFκB-NS were used in the following studies.

The balloon surface of the catheter was negatively charged, associated with the hydrophilic coating by methyl vinyl ether-maleic anhydride copolymer. This polymer is widely used for medical devices, such as guide wires and catheters, to produce a more slippery surface in a wet condition. In contrast, PLGA NS could be electrostatically bound to the balloon surface without additional agents (Figure 1B). As a result, NFκB-NS were homogenously coated on the surface of the balloon (Figure 1C and 1D). A total of 5.4 mg NFκB-NS containing 2.3% decoy ODN (125 μg, 0.16 μg/mm²) was coated on balloons with a diameter of 5 mm, and 2.8 mg NFκB-NS containing 4.5% decoy ODN (126 μg, 0.45 μg/mm²) was coated on balloons with a diameter of 3 mm.

Inhibitory Effects of NFκB DCB Catheter on Neointimal Formation After Angioplasty

To investigate the therapeutic effects of the NFκB DCB catheter in vivo, angioplasty was performed in 2 types of vascular injury models in rabbits. There was no difference in the handling, flexibility, and short-term tolerance between NFκB-NS–coated and uncoated balloon catheters. No sign of thrombotic events was observed during and after balloon expansion. In normal abdominal aorta, the short-term contact of the H-loaded PLGA NS (H-NS)–coated balloon with the arterial wall allowed rapid transfer of H-NS. Because administration of H-NS into the target site was performed within 40 seconds with this delivery system (Figure 2A), a 1-minute inflation time was selected for angioplasty. In addition, an increase of
radioactivity in the aorta was also detected ≥7 days after balloon expansion (Figure 2A).

Initially, we evaluated the preventive effect of the NFκB DCB on neointimal formation in single vascular injury of the rabbit aorta, in which angioplasty was performed immediately after endothelial denudation. A distribution study using a fluorescent isothiocyanate-labeled PLGA NS (FITC-NS)-coated balloon revealed that fluorescence could be detected in the inner media of the total luminal surface after balloon expansion (Figure 2B). Elastic van Gieson’s staining demonstrated that angioplasty with an uncoated balloon was associated with marked neointimal hyperplasia at 4 weeks after treatment. In contrast, usage of an NFκB DCB inhibited the progression of neointimal hyperplasia and reduced the intima-to-media ratio as compared with control (Figure 2C).

As successful results were obtained with the NFκB DCB catheter in a single-injury model, we further investigated the efficacy of this catheter in a double-injury model in rabbits as a clinically relevant model. After confirmation of lesion formation in injured carotid arteries by angiography at 4 weeks after initial endothelial denudation, balloon angioplasty was performed. No significant differences were shown in diameter stenosis rates before angioplasty among 3 groups (Table I and Figure I in the Data Supplement). Angioplasty using an fluorescent isothiocyanate-NS-coated balloon showed successful transfer of fluorescent isothiocyanate-NS in the neointima at 7 days after balloon expansion at the target site (Figure 3A). Moreover, immunofluorescent staining revealed that a part of fluorescent isothiocyanate-NS was detected in the migrating macrophages and α-smooth muscle actin-positive cells in the neointima (Figure 3A). Electrophoretic mobility shift assay demonstrated that binding activity of NFκB was markedly increased in the arterial wall in control at 4 weeks after angioplasty. However, NFκB activity was significantly inhibited by local application of NFκB-NS, but not scrambled-NS (Figure 3B). Consistently, treatment with the NFκB DCB significantly inhibited neointimal hyperplasia and reduced the intima-to-media ratio as compared with control or scrambled DCB treatment at 4 weeks after angioplasty. In addition, morphological study demonstrated a significant increase in lumen area in vessels treated with NFκB-NS (Figure 4A and 4B).

Molecular Mechanisms Underlying Prevention of Neointimal Formation by NFκB DCB

Given the beneficial effects of the NFκB DCB catheter on neointimal formation, we further investigated the molecular mechanisms. Initially, the anti-inflammatory effect of NFκB-NS was evaluated by immunohistochemical staining. Numerous macrophages infiltrated the neointima of vessels transfected with scrambled-NS or control, whereas macrophage recruitment was significantly inhibited by treatment with NFκB-NS (Figure 5A). Infiltration of inflammatory cells is mainly controlled by adhesion molecules and chemotactic factors, and the expression levels of the monocyte chemoattractant protein-1, vascular cell adhesion molecule-1, and CC chemokine ligand 4 genes were increased in the control and scrambled-NS groups. However, a significant reduction in gene expression of these factors was observed by treatment.
with NFκB-NS at 1 week after angioplasty (Figure 5B). Furthermore, immunofluorescent staining also demonstrated that the expression of vascular cell adhesion molecule-1 was significantly inhibited by treatment with NFκB-NS (Figure II in the Data Supplement).

Next, we investigated the proliferative activity of VSMC in the neointima. Proliferating cell nuclear antigen was detected in α-smooth muscle actin-positive cells in the neointima in sections of vessels with scrambled-NS transfer (Figure 6A). In addition, vessels treated with scrambled-NS or control showed markedly increased expression of proliferating cell nuclear antigen and cyclin A, whereas treatment with NFκB-NS significantly inhibited the expression of these proteins (Figure 6B). Although α-smooth muscle actin-positive cells and collagen deposition were markedly found within the neointima in specimens of control and scrambled-NS transfer, the ratio of α-smooth muscle actin-positive cells or collagen deposition by comparing total fractional area of the cross section was not significantly deference in each group (Figure III and IV in the Data Supplement).

Finally, re-endothelialization of the luminal surface at the site of balloon injury was evaluated. Immunohistochemical staining demonstrated that restoration of the endothelial monolayer was significantly accelerated by treatment with NFκB-NS as compared with control or scrambled-NS (Figure 7A). In addition, although the expression of phosphorylated Bcl-2 was shown in endothelial cells in normal arteries, treatment with scrambled-NS reduced phosphorylated Bcl-2 expression. In contrast, local application of NFκB-NS resulted in the preservation or enhanced expression of phosphorylated Bcl-2 in endothelial cells (Figure 7B).

To consider the clinical utility of the NFκB DCB catheter, laboratory data were examined before and after angioplasty. As expected, local application of NFκB-NS did not result in any acute toxicity, and there was no significant difference in body weight between rabbits treated with NFκB-NS and those treated with scrambled-NS or control at 4 weeks after angioplasty (data not shown). No alteration in hematologic or serum chemical findings was induced at 1 week after angioplasty by NFκB DCB (Table II in the Data Supplement). The systemic distribution study following 3H-NS–coated balloon treatment demonstrated that radioactivity in plasma dropped quickly after balloon expansion. In addition, radioactivity in the kidney and liver was gradually decreased at 24 hours after angioplasty (Figure V in the Data Supplement).

Discussion

Here, we demonstrated that short-term transfer of NFκB decoy ODN using an angioplasty balloon catheter resulted in potent therapeutic effects to prevent neointimal formation in arteries with acute injury, as well as in a double-balloon injury model of rabbits, through inhibition of inflammatory response and proliferation of VSMC and acceleration of re-endothelialization.

Instead of DES, the development of drug-coated balloon catheters is the center of interest because DES necessitate long-term antiplatelet therapy in spite of a marked decrease in
restenosis. However, drug-coated balloon catheters seem to be more problematic because the period of drug administration in the target site is too short, probably <60 seconds. In addition, although naked phosphorothioate ODN in cells could work over 2 weeks, the clinical application of ODN-based technology is limited because of the slow uptake across the cell membrane and rapid degeneration of ODN before internalization in cells, leading to an insufficient concentration of ODN and inappropriate duration for treatment. To overcome these problems, we used a nanoparticle-based delivery system. PLGA and chitosan are natural polymers and are thought to be efficient drug carriers because of their low immunogenicity, high safety, and biocompatibility. Importantly, PLGA has been used in clinical devices, and low cytotoxicity of chitosan-modified PLGA NS was confirmed by several basic studies.17 By entrapping them in the PLGA nano-matrix, decoy ODN were protected against enzymatic degeneration and obtained a controlled release profile in vivo. In addition, the positively charged chitosan allowed the absorption of anionic decoy ODN onto the particle surface by means of ionic interaction and inhibited leakage of decoy ODN from the nano-matrix before releasing ODN in the cytoplasm. Furthermore, the modified emulsion solvent diffusion method for preparation of NFkB-NS inhibited ODN degradation by mechanical stress during preparation and yielded NFkB-NS with a spherical shape, smooth surface, and uniform diameter.16

A biphasic release profile of decoy ODN is also important to prevent restenosis. Initial rapid release of ODN was mainly attributed to the absorbed decoy ODN on chitosan, and sustained release was associated with hydrolytic degradation of PLGA. Regulation of the initial inappropriate gene expression at the time of injury is thought to lead to long-term stabilization of injured vessels. However, adventitial myofibroblasts, progenitor cells, and stem cells from bone marrow accumulate in injured vessels and participate in neointimal formation at a later time. Therefore, prolonged retention of agents in injured vessels might also be necessary to regulate neointimal growth. In contrast to the hydrophilic property of naked decoy ODN, NFkB-NS showed high lipophilicity by forming an ionic complex between ODN and chitosan on the particle surface,16 resulting in effective local retention in target vessels.

After administration in the arterial wall, internalization of NFkB-NS into target cells is critical for transcriptional regulation. Although nanoparticles have relatively high penetration into tissues and cells because of their subcellular size and mobility, surface modification with chitosan can also act as an intracellular delivery system. As both decoy ODN and the cell membrane are negatively charged, it is difficult for decoy ODN to pass through the cell membrane passively. In contrast, as NFkB-NS showed a positive charge by coating with chitosan, NFkB-NS can easily interact with the negatively charged cell membrane, leading to an increase of cellular uptake.18 After internalization within the cytoplasm by endocytosis, PLGA NS is thought to have an efficient endosomal escape system, the proton-sponge mechanism.20 Therefore, this delivery system could induce a sufficient dose of decoy ODN in target cells and mediate stability of the biological

Figure 6. Inhibition of proliferative activity in vascular smooth muscle cells (VSMC) by local administration of nuclear factor-kappaB nanoparticles (NFkB-NS). A, Double immunofluorescent staining for α-smooth muscle actin and proliferating cell nuclear antigen in tissue sections of carotid arteries with scrambled-NS transfer at 1 week after angioplasty. B, Western blotting of proliferating cell nuclear antigen, cyclin D1, and cyclin A. β-actin was used as the internal control for normalization (n=5 per group). §P<0.05 vs scrambled; *P<0.05 vs sham; #P<0.05 vs control and scrambled. Scale bar in A, 20 μm.
environment during the critical period of neointimal formation after angioplasty.

In addition to an intracellular drug delivery method, the development of an efficient site-specific drug application system is needed for clinical utility. A drug-coated balloon catheter is simple to use and allows localization of therapeutic agents to the target vessel wall during angioplasty, with minimized vascular injury. To achieve high transfection efficiency, we used an electrostatic interaction for coating NFκB-NS on the balloon surface. Although naked decoy ODN are easily dissolved in the bloodstream through their hydrophilic property, cationic NFκB-NS have a hydrophobic property and could bind to the negatively charged balloon surface. This system would enhance the loading capacity of NFκB-NS on the balloon surface without the use of carrying agents and introduce only NFκB-NS into the arterial wall during balloon expansion for 1 minute, resulting in significant inhibition of the binding activity of NFκB. These observations indicate that this local delivery system was an effective approach to introduce ODN in short-term contact with target vessels.

To clarify the efficacy of the NFκB DCB catheter in vivo, we performed balloon angioplasty in 2 types of vascular injury models in the rabbit. In accordance with the effective inhibition of NFκB activity, local application of NFκB-NS inhibited neointimal formation and lumen loss after balloon angioplasty. The beneficial effects of NFκB-NS were associated with regulation of multiple phenomena. First, treatment with NFκB-NS mediated anti-inflammatory effects in target vessels. Treatment with NFκB-NS inhibited the recruitment of macrophages in the neointima through the suppression of MCP-1, VCAM-1, and CCL4 gene expression. Neointimal formation is viewed as a chronic inflammatory disease, and NFκB is a critical transcription factor regulating transactivation of proinflammatory genes, leading to accumulation of inflammatory cells in the injured artery. Second, treatment with NFκB-NS suppressed the proliferative activity of VSMC at the site of angioplasty. As neointimal hyperplasia is characterized by VSMC proliferation and migration, VSMC growth is thought to be a potent therapeutic target. Although the transcription factor, E2F, is well known to regulate cell cycle progression, NFκB also plays an important role in the transcription of cell cycle regulatory genes.

Third, another important therapeutic effect of NFκB-NS is protection against endothelial cell death caused by several types of damage during and after balloon injury. The intervention procedure causes endothelial denudation or dysfunction, and delayed re-endothelialization contributes neointimal formation and thrombotic complications. In addition, marked endothelial cell apoptosis was observed in atherosclerotic-prone lesions. Interestingly, administration of NFκB-NS accelerated restoration of the endothelial monolayer in the early phase after angioplasty, through preservation or enhanced expression of phosphorylated Bcl-2 in endothelial cells. Our previous data demonstrated that the inhibition of NFκB activity suppressed endothelial cell death through enhanced expression of Bcl-2 under hypoxic conditions or oxidative stress.

![Figure 7](http://www.ahajournals.org/doi/fig/10.1161/CIRCINTERVENTIONS.114.001340)

**Figure 7.** Restoration of endothelial monolayer by local administration of nuclear factor-kappaB nanosphere (NFκB-NS). **A**, Typical example of immunohistochemical staining of CD31 and percentage of luminal surface covered by CD31-positive cells at 4 weeks after angioplasty (n=5 per group). **B**, Double immunofluorescent staining for CD31 and phosphorylated Bcl-2 and percentage of p-Bcl-2-positive cells in CD31-positive cells at 1 week after angioplasty (n=5 per group). p-Bcl-2; phosphorylated B-cell lymphoma 2. *P*<0.05 vs sham; #P<0.05 vs control and Scrambled. Scale bar in **A** left panel, 500 μm; right panels, 200 μm; in **B**, 50 μm.
stress in vitro. In addition, expression of Bcl-2 increased proliferation and migration of endothelial cells, independent of its survival activity. Recent studies also demonstrated that inhibition of NFκB activity decreased apoptosis of endothelial progenitor cells. Therefore, antiapoptotic effect of NFκB-NS is one of the mechanisms underlying accelerated re-endothelialization. Recently, the beneficial effect of a paclitaxel-coated balloon catheter on neointimal formation was reported in clinical studies, including patients with in-stent restenosis and femoro-popliteal disease. However, as the antiproliferative effect of paclitaxel is mainly caused by arrest of microtubule function by binding to the β subunit of tubulin, paclitaxel also impaired endothelial cell proliferation, leading to increased risk of thrombosis and delayed vascular healing. In addition, paclitaxel induced programmed cell death in endothelial cells, through inhibition of Bcl-2 expression. Therefore, the protective effect of NFκB-NS on endothelial cells might add a potent therapeutic advantage to prevent restenosis compared to other antiproliferative agents.

Finally, the safety of NFκB-NS is a key concern in clinical application. A part of NFκB-NS on the balloon surface dissolved in the blood stream during balloon expansion, and nanoparticles in blood would be removed by renal excretion or uptake by proximal tubule epithelial cells in the kidney and reticuloendothelial system, including Kupffer cells in the liver. However, our data demonstrated no adverse effects associated with the NFκB DCB catheter, and rapid clearance of 1H-NS was observed from these organs and plasma. These data support the safety of the use of NFκB DCB catheters in clinical settings.

This study has several limitations. We used 2 types of vascular injury model in rabbits to investigate the efficacy of NFκB-DCB. Although the double-injury model showed significant lesion formation in injured arteries, these lesions are variable in morphology and severity. In addition, the transfection efficiency to disease artery would worsen in the clinical setting because human arteries as target of angioplasty are usually associated with atherosclerotic change. Therefore, further studies are necessary to clarify the efficacy of NFκB-DCB in humans.

In conclusion, the present study demonstrated the efficacy and safety of balloon angioplasty using an NFκB DCB catheter in rabbit models. This novel drug delivery system could achieve high transfection efficiency and controlled release of NFκB decoy ODN into target vessels without delayed vascular healing or systemic adverse effects. Because balloon angioplasty cannot prevent elastic recoil and dissection at the site of intervention, NFκB-DCB is not well suited to treat de novo coronary stenosis. However, this balloon catheter system has the potential for treating stenotic lesions in which other endovascular techniques are not suited or technically challenging, as well as in-stent restenosis.

Acknowledgments

We greatly thank Hikuzi Hamada for expert technical assistance.

Sources of Funding

This work was partially supported by a Grant-in-Aid from the Japan Innovation Promotion Program and the Translational Research Promotion Project of the New Energy and Industrial Technology Development Organization.

Disclosures

Dr Morishita has stocks for AnGes MG. The other authors report no conflicts.

References


Prevention of Neointimal Formation After Angioplasty Using Nuclear Factor-κB Decoy Oligodeoxynucleotide-Coated Balloon Catheter in Rabbit Model
Takashi Miyake, Shinya Ihara, Tetsuo Miyake, Yusuke Tsukada, Hajime Watanabe, Hiroaki Matsuda, Hideki Kiguchi, Hiroyuki Tsujimoto, Hironori Nakagami and Ryuichi Morishita

Circ Cardiovasc Interv. 2014;7:787-796; originally published online November 18, 2014; doi: 10.1161/CIRCINTERVENTIONS.114.001522
Circulation: Cardiovascular Interventions is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-7640. Online ISSN: 1941-7632

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circinterventions.ahajournals.org/content/7/6/787

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Interventions can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Interventions is online at:
http://circinterventions.ahajournals.org//subscriptions/