Transcatheter Delivery of c-myc Antisense Oligomers Reduces Neointimal Formation in a Porcine Model of Coronary Artery Balloon Injury

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Background Smooth muscle cell proliferation and extracellular matrix accumulation are the principal mechanisms leading to vascular restenosis. We have previously demonstrated the growth-inhibitory effect of antisense oligomers targeting the c-myc proto-oncogene in human smooth muscle cells. The goal of this study was to investigate whether c-myc antisense oligomers reduce neointimal formation in balloon-dened porcine coronary arteries.

Methods and Results First, type I collagen synthesis, which reflects synthetic function, was markedly reduced following c-myc antisense oligomers in porcine vascular smooth muscle cells independent of the growth inhibition. These effects in vitro provided the rationale for assessing c-myc antisense oligomers in the prevention of neointima in vivo. Second, the efficiency of single transcatheter delivery of oligomers into denuded porcine coronary arteries was determined. Despite rapid plasma clearance following local delivery, oligomers persisted at the site of injection for at least 3 days, exceeding by severalfold their concentration in peripheral organs. Third, morphometric analyses were carried out in balloon-denuded coronary arteries at 1 month after transcatheter c-myc antisense oligomer administration. Maximal neointimal area was reduced from 0.80±0.17 mm² in the control group (n=12) to 0.24±0.06 mm² in the antisense-treated group (n=13, P<.01). Likewise, a significant reduction in maximal neointimal thickness was observed in the antisense-treated group (P<.01). These changes in vascular remodeling following denuding injury resulted in an increase in residual lumen from 64±6% in the control group to 81±5% in the antisense-treated group (P<.05).

Conclusions (1) Single transcatheter administration allowed for endoluminal delivery of oligomers to the site of coronary arterial injury. (2) C-myc antisense oligomers reduced the formation of neointima in denuded coronary arteries, implying a therapeutic potential of this approach for the prevention of coronary restenosis. (3) It is postulated that the c-myc proto-oncogene is involved in the process of vascular remodeling, regulating smooth muscle cell proliferation and extracellular matrix synthesis. (Circulation. 1994;90:944-951.)

Key Words c-myc • proto-oncogenes • smooth muscle cells • antisense oligomers • extracellular matrix • restenosis

Vascular smooth muscle cells (VSMCs) are highly differentiated cells that modulate their phenotype and dedifferentiate under certain pathological conditions, contributing to the development of atherosclerosis and restenosis following vascular injury related to catheter-based interventions. Three major phenotypic changes have been identified regarding VSMCs after denuding injury in normal vessels; they involve migration of VSMCs from the media to the intima, their proliferation, and enhanced synthesis of extracellular matrix. The inhibition of VSMC proliferation using antisense oligodeoxynucleotides (ODNs) has suggested a potential therapeutic role of this approach in the prevention of coronary restenosis. However, the complexity of human vascular lesions and diverse biological functions of VSMCs resulting in excessive collagen accumulation after vascular injury, raise the question of whether antisense strategies aimed at growth control alone are sufficient to prevent neointimal formation.

The use of antisense ODNs for the prevention of coronary restenosis is also hampered by the difficulty in their transfer to target tissue. Previous studies with antisense ODNs have been carried out in a rat carotid artery model. Although adventitial and surgical routes of ODN delivery have been effective in the reduction of neointimal formation, they have a limited applicability in the prevention of coronary restenosis. Local transcatheter administration as an alternative for ODN transfer to the site of angioplasty is more practical, but it is currently limited by unproven efficiency as well as by rapidly developing myocardial ischemia if prolonged application is required. Therefore, the paucity of studies using a clinically applicable protocol to deliver antisense ODNs following coronary denudation requires the evaluation of this novel approach with an endoluminal route of administration in the coronary vasculature.

The present study has attempted to address the above unresolved issues using antisense ODNs directed against the c-myc proto-oncogene because of its involvement in the regulation of VSMC proliferation. Accordingly, first in porcine VSMCs in vitro, we have
determined that c-myc antisense ODNs independently inhibited not only cell proliferation but also synthetic function. These results provided the rationale for further testing of c-myc antisense ODNs in the cardiovascular system in vivo. Using a transcatheter route, we demonstrated the feasibility of local endoluminal delivery of ODNs to the site of coronary denudation. Finally, to determine the biological importance of these effects, we used a single transcatheter administration of antisense ODNs achieving an effective inhibition of neointimal formation in denuded coronary arteries in a porcine model in vivo.

**Methods**

**Cell Culture**

Porcine VSMCs were isolated from the aortae of domestic swine using an explant method. The explants were placed into a tissue culture dish containing Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mM/L glutamine (10% FBS-DMEM). The cultures were maintained at 37°C in a humidified incubator with 5% carbon dioxide. The cells exhibited typical morphological characteristics of VSMCs, ie, spindle shape and “hill-and-valley” pattern. The identification of VSMCs was further confirmed by smooth muscle α-actin staining.

**Synthesis of Oligomers**

15-Mer ODNs were synthesized on an Applied Biosystems model 394 high-throughput DNA synthesizer (Applied Biosystems). The ODNs were lyophilized, resuspended in PBS, and quantified by spectrophotometry. Phosphorothioate ODNs from the translation initiation region of the human c-myc gene were used in this study. The ODN sequences were as follows: sense (5’ TTGCAATCCTCGCTA 3’), 4-bp mismatched (5’ AACGTGAGTTCGAG 3’), scrambled (5’ GAACCGAGACGGTTT 3’), and antisense (5’ AACCTGGAGGGGACAT 3’).

**Growth Assay**

Porcine (passages 2 through 6) VSMCs were seeded in 10% FBS-DMEM at a density of 5000 cells/cm². One day after plating, cells were arrested in 0.5% FBS-DMEM for 48 hours. Cells grown with 10% FBS-DMEM were defined as control (FBS-DMEM). The ODNs were added at the time of stimulation. Three days later, cells were trypsinized and counted on a Coulter counter (Coulter Electronics Corp.). Each experiment was carried out in triplicate and repeated three times on different occasions. Cell viability following treatment with ODNs was assessed using trypan blue exclusion.

**Assessment of Type I Collagen by Western Blot**

Porcine VSMCs were plated at 25 000 cells/cm² in 10% FBS-DMEM. After the cells reached confluence and became density arrested, medium was replaced with fresh 10% FBS-DMEM containing ascorbic acid (50 μg/mL) with or without ODNs. The culture medium was collected 24 hours afterward, and the cell number was counted. The samples were digested with pepsin (1 mg/mL) in 0.5 mol/L acetic acid overnight at 4°C. They were then lyophilized and resuspended in a buffer containing 1% SDS and 5% 2-mercaptoethanol. After being boiling for 5 minutes, the samples were electrophoresed in 7.5% (wt/vol) premade SDS polyacrylamide gels (BioRad). The fractionated proteins were then electrotransferred onto PolyScreen PVDF membranes (DuPont NEN) in transfer buffer containing 0.192 mol/L glycine, 25 mol/L Trizma base, 0.01% SDS, and 20% methanol. The blots were then blocked in 5% nonfat milk in PBS and sequentially incubated with biotin-labeled anti-human type I collagen antibodies, which react with triple helical domain of the human type I collagen (Southern Biotechnology Associates, Inc), streptavidin-alkaline phosphatase (Boehringer Mannheim), and chemiluminescence reagent (DuPont NEN) as described by the manufacturer. The membranes were exposed to Kodak X-Omat film and quantified by laser densitometry. Densitometric values were normalized by cell number, and the percent inhibition of collagen was calculated against control samples without the addition of ODNs.

**Animal Preparation and ODN Delivery In Vivo**

Domestic crossbred pigs (Sus scrofa) weighing from 23 to 31 kg were premedicated with aspirin (650 mg PO) before the study. General anesthesia consisted of an intramuscular injection of ketamine (20 mg/kg) and xylazine (4 mg/kg). Additional doses of anesthesia were given intravenously throughout the experiment. The right external carotid artery was surgically exposed, and heparin (10 000 U) was administered intravenously. Using an 8F SAL 1 guiding catheter (Medtronic Interventional Vascular, Inc), the coronary ostia were cannulated under fluoroscopic guidance, and intracoronary nitroglycerin was administered (100 μg). Afterward, selected segments of coronary arteries were denuded using an oversized balloon (3.5 to 4.0 mm), which was inflated three times (6 to 10 atm) for 30 seconds. This procedure results in the induction of neointimal lesions containing predominantly VSMCs. Animals were randomized to the control group (n=12) receiving either vehicle injection (saline, n=3) or sense ODNs (n=9, 1 mg per vessel) or the treated group receiving c-myc antisense ODNs (n=13, 1 mg per vessel). The oversized balloon was replaced with a porcous balloon (3.0 to 3.5 mm, kindly provided by USCI, Inc), which was advanced to the site of coronary denudation. All intraluminal injections were carried out under 4 atm of pressure within 5 minutes of denuding injury. The total volume of injectate administered via a porcous balloon was 2 mL per vessel. Then, final coronary angiograms were recorded to confirm the continued vessel patency. The catheters were removed, surgical wounds were repaired, and the animals were allowed to recover. At the indicated times, the animals were given a lethal dose of sodium pentobarbital (100 mg/kg IV).

**Biodistribution of ODNs In Vivo**

15-Mer ODNs were labeled with 35S at each internucleotide linkage and mixed with unlabeled ODNs to achieve a desired concentration (1 mg per vessel, 3.8 μCi). Single transcatheter delivery into denuded coronary arteries was carried out as described above. Afterward, blood samples, injured and non-injured coronary vessels, and peripheral organs remote from the site of injection were collected at various time points as indicated in the text. Tissue samples were rapidly frozen in liquid nitrogen, crushed into fine powder, and then homogenized in lysis buffer (25 mmol/L triphosphate [pH 7.8], 2 mmol/L DTT, 1,2-diaminocyclohexane-N,N,N',N'-tetra-acetic acid, 10% glycerol, 1% Triton X-100, and 50 μg/mL proteinase K). The radioactivity of each sample was counted in a scintillation counter (Pharmacia LKB Biotechnology), and the ODN concentration was quantified according to a standard prepared with a known quantity of 35S-labeled ODNs.

**Morphometric Analyses**

The heart was rapidly removed and perfused antegradeably with 10% buffered formalin for 15 hours. Based on previously recorded coronary angiograms, injured portions of each coronary artery were identified and removed. After dehydration in graded ethanol, the samples were embedded in paraffin and cross sectioned. Serial 5-μm-thick sections at 2-mm increments were stained with Verhoeff-van Gieson stain. The most severe luminal narrowing in denuded segments was identified based on the review of 10 sections per vessel. Morphometric analyses were performed on the sections demonstrating the most severe luminal narrowing as described previously.
The maximal neointimal area (mm²), neointimal thickness (mm), and residual lumen ([luminal area/luminal + neointimal areas] × 100) were analyzed using a computerized imaging system (Advanced Imaging Concepts, Inc). To compare the severity of medial disruption between the groups, a modified histological injury score was used. Animals were excluded if no injury to the internal elastic lamina was noted. All histological measurements were performed by two observers blinded to the treatment assignment with the interobserver variability remaining within 10%.

Statistical Analysis

Data are expressed as mean±SEM. The statistical significance of the growth inhibition in vitro was determined using ANOVA. Morphometric data were compared using unpaired Student’s t test. A value of P<.05 was considered significant.

Results

Inhibition of Growth and Type I Collagen Synthesis

To determine potential usefulness of c-myc antisense ODNs in the prevention of neointimal formation, VSMC growth and type I collagen synthesis were evaluated following antisense ODNs in porcine VSMCs before in vivo studies. C-myc antisense ODNs produced a significant growth-inhibitory effect, whereas sense, mismatched, or scrambled ODNs exerted no effect on cell proliferation (Fig 1). The inhibitory effect was dose dependent. The treated VSMCs demonstrated normal morphology with more than 95% of cells remaining viable at the tested dose range as determined by trypan blue exclusion assay.

In addition, type I collagen produced by VSMCs was quantified and compared following various ODN treatments. The experiments were carried out in density-arrested (ie, confluent) VSMCs to exclude the effect of cell growth on collagen synthesis. Control ODNs (ie, sense, 4-bp mismatched, and scrambled sequences) showed no effect on type I collagen synthesis compared with cells without the addition of ODNs. In contrast, collagen synthesis was markedly reduced by c-myc antisense ODNs (Fig 2). The inhibition was dose dependent, and type I collagen synthesis was decreased by more than 90% at a dose of 16 μmol/L. No cell toxicity was noted by trypan blue exclusion assay, and all cultures were metabolically active as indicated by 35S-methionine incorporation in the treatment groups (data not shown). The reduction of type I collagen synthesis, which occurred in nonproliferating, density-arrested VSMCs, was independent of the growth-inhibitory effect of c-myc antisense ODNs.

Accordingly, c-myc antisense oligomers exerted inhibitory effects on two major characteristics of VSMCs, ie, cell proliferation and collagen synthesis, which both are implicated in the pathogenesis of neointimal formation. The above in vitro studies provided the rationale for the testing of c-myc antisense ODNs in a porcine model of coronary denudation.

Biodistribution of ODNs With Local Transcatheter Delivery

An important step toward the application of antisense ODNs for the prevention of neointimal formation is to assess the ability to deliver ODNs to the site of denudation. Accordingly, to determine the efficiency of coronary local transcatheter delivery of ODNs into the coronary arterial wall, pharmacokinetics and organ distribution of 35S-labeled ODNs were determined. Fig 3 demonstrates plasma levels of 35S-labeled ODNs after local administration into the coronary vasculature. Plasma levels were highest at the time of first blood sampling, ie, 5 minutes following delivery, and rapidly
declined thereafter. Pharmacokinetic data and biodistribution of ODNs are shown in Table 1.

The intramural concentrations of ODNs were determined in coronary arteries after transcatheter administration (1 mg). As can be seen in Table 2, they were 23.4 μg/g at 30 minutes, 18.2 μg/g at day 1, and 51.0 μg/g at day 3, whereas in remote (ie, noninjured) vessels the amount was within the background level. Therefore, local injection using a transcatheter approach provided sustained levels of ODNs in coronary arteries for at least 3 days despite rapid ODN clearance from plasma. The ODN distribution in peripheral organs indicated transient accumulation of ODNs in the kidney and liver (Table 2). However, the ODN concentration was 2- to 69-fold and 4- to 73-fold higher at the site of injection than levels found in remote organs at 30 minutes and at 24 hours following intramural administration, respectively. These results demonstrated that the transcatheter route of ODN delivery provided sustained levels of antisense at the site of denudation.

Neointimal Formation

Because c-myc antisense ODNs inhibited VSMC growth and type I collagen synthesis, both of which are involved in neointimal formation, we sought to determine whether a single endoluminal delivery of ODNs (1 mg per vessel) modifies vessel wall response to injury. The delivery time was 22±1 seconds (range, 16 to 30 seconds) with all coronary arteries remaining angiographically patent after the removal of a porous balloon. There was no difference between the control and antisense-treated groups regarding the size of denuding and porous balloons used. Computerized morphometry was carried out on the coronary arterial section demonstrating the largest neointimal lesion as identified by histopathological analysis. The control and antisense-treated vessels were comparable as reflected by similar medial areas. No differences in morphometric parameter

Table 1. Plasma Pharmacokinetics and Distribution of Oligodeoxynucleotides After Transcatheter Delivery Into the Coronary Vasculature (1 mg per Vessel)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1/2 α distribution</td>
<td>0.2 h</td>
</tr>
<tr>
<td>T1/2 β elimination</td>
<td>198 h</td>
</tr>
<tr>
<td>Volume of distribution</td>
<td>0.25 L/kg</td>
</tr>
<tr>
<td>Area under the curve</td>
<td>31.5 μg · h/mL</td>
</tr>
</tbody>
</table>

Table 2. Distribution of Oligodeoxynucleotides in the Peripheral Organs After Transcatheter Delivery into the Coronary Vasculature (1 mg per Vessel)

<table>
<thead>
<tr>
<th>Organ</th>
<th>30 min</th>
<th>1 d</th>
<th>3 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary artery</td>
<td>23.40</td>
<td>18.20</td>
<td>51.00</td>
</tr>
<tr>
<td>Kidney</td>
<td>10.12</td>
<td>4.80</td>
<td>0.70</td>
</tr>
<tr>
<td>Liver</td>
<td>2.18</td>
<td>2.39</td>
<td>1.71</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>0.45</td>
<td>0.33</td>
<td>1.14</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.34</td>
<td>0.25</td>
<td>0.29</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.53</td>
<td>0.51</td>
<td>0.48</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>0.37</td>
<td>0.38</td>
<td>0.46</td>
</tr>
<tr>
<td>Lung</td>
<td>0.49</td>
<td>0.42</td>
<td>0.43</td>
</tr>
</tbody>
</table>

All values are given in μg/g; 30 min, 1 d, and 3 d refer to time after oligodeoxynucleotide delivery when the organs were harvested.

In contrast, there was a significant reduction in the maximal neointimal area from 0.80±0.17 mm² in the control group (n=12) to 0.24±0.06 mm² in animals receiving c-myc antisense oligomers (n=13, P<.01, Fig 4). Likewise, the maximal neointimal thickness was 0.48±0.09 mm in the control group, whereas in the c-myc antisense–treated group it was 0.20±0.04 mm (P<.01, Fig 4). Thus, a single transcatheter administration of c-myc antisense ODNs produced 70% and 58% reductions in neointimal area and thickness, respectively. These changes resulted in the improvement in residual coronary lumen from 64±6% in the control group to 81±5% in the antisense-treated group (P<.05).

Because neointimal formation is related to the severity of medial injury, we have compared histological

Fig 3. Plot showing mean plasma concentration of oligodeoxynucleotides after local transcatheter delivery into coronary arteries (n=4).

Fig 4. Bar graphs showing results of morphometric analysis at 1 month after c-myc sense or antisense oligodeoxynucleotide administration into denuded porcine coronary arteries. Top, Maximal neointimal area in the control (n=12) and c-myc antisense–treated groups (n=13, P<.01). Bottom, Maximal neointimal thickness in the control (n=12) and c-myc antisense–treated groups (n=13, P<.01).
injury score (Table 3) between the control and the antisense-treated groups. Mean injury score was 2.7±0.4 in the control group and 2.0±0.3 in the antisense-treated group (P=NS). This demonstrated comparable vascular injury, indicating that the observed reduction in neointimal formation could not be attributed to differences in the severity of denudation. When the maximal neointimal area was further analyzed as a continuous function of histological injury, c-myc antisense oligomers reduced neointimal formation even in those vessels with more advanced medial disruption (slopes: P=.001, Fig 5). Fig 6 illustrates reductions in neointimal formation in porcine coronary arteries following c-myc antisense ODNs compared with the sense-treated animals.

Discussion

The c-myc nuclear proto-oncogene is a ubiquitous phosphoprotein that is believed to regulate gene expression on transcriptional17 and posttranscriptional18 levels resulting in cell proliferation and their dedifferentiation. VSMCs derived from atherosclerotic lesions exhibit increased expression of c-myc,19 and balloon injury itself provides a powerful stimulus for an increase in c-myc expression.20,21 This study presents the evidence for the inhibition of two different characteristics of VSMCs, ie, growth and synthetic function, in response to c-myc antisense ODNs. These findings were paralleled by a significant reduction in neointimal formation after a single transcatheter administration of c-myc antisense ODNs in a porcine model of coronary arterial denudation. Therefore, the c-myc nuclear proto-oncogene appears to have an important role contributing to neointimal formation.

Synthetic Phenotype of VSMCs in the Process of Vascular Restenosis

VSMCs are responsible for a broad spectrum of functions including maintaining vascular tone as well as the repair and remodeling of the vessel wall. To accomplish such multiple tasks, VSMCs have to modulate their phenotype.1,5,22,23 Therefore, at one end of the spectrum are VSMCs whose primary function is contraction (ie, “contractile” phenotype). At the other end are VSMCs that in response to an altered environment are able to modulate to a “synthetic” phenotype exhibiting greatly increased synthetic and proliferative capacity. A number of studies have demonstrated a heterogeneous population of VSMCs, including cells exhibiting “synthetic” phenotype, in human atherosclerotic and restenotic lesions.1,24,25 Recently, however, atherectomy specimens provided conflicting results regarding the extent of cell proliferation in restenotic lesions in patients with underlying atherosclerotic plaque.26,27 A low level of cell proliferation in some restenotic samples requires careful evaluation of other pathomechanisms of restenosis. If VSMCs undergo multiple cell doublings as in the process of atherogenesis, their ability to divide may be limited.1,22 albeit they continue to produce matrix proteins on exposure to growth factors.1,28 An inverse relation between VSMC growth and collagen synthesis has been demonstrated in cultured synthetic VSMCs.29-30 These observations provide the rationale for the development of therapeutic strategies addressing not only cell proliferation but also collagen synthesis for the prevention of vascular restenosis. The present study has demonstrated that antisense ODNs targeting the c-myc proto-oncogene markedly inhibited both VSMC proliferation and type I collagen synthesis. The reduction in collagen synthesis was independent of growth-inhibitory effects following c-myc antisense ODNs, suggesting a novel function of the c-myc proto-oncogene in the regulation of extracellular matrix formation. A sustained expression of the c-myc proto-oncogene in confluent cells in the presence of growth factors31 provided a target for antisense ODNs. An exact mechanism involving c-myc in the regulation of collagen synthesis remains to be determined. Our preliminary studies suggest a posttranscriptional regulation with the degradation of type I collagen unaffected by c-myc ODN treatment (unpublished data).

Application of Antisense ODNs in Vascular Biology

The modification of a disease process using antisense strategies requires demonstration of their efficacy in a complex in vivo environment. Although “antisense” recognition of the complementary transcripts of the target gene is based on a classic Watson-Crick base pairing,32,33 the ultimate biological effects of antisense DNA will also depend on the relevance of a molecular target and the pattern of its expression. The precise mechanisms involved in the regulation of gene expression by antisense ODNs include translational arrest, the cleavage of mRNA by RNase-H activity, the inhibition of RNA maturation, and its transport out of the nucleus.34-36 The use of an antisense approach may provide several advantages in vascular biology, including preferential targeting of synthetic VSMCs expressing a gene of interest. The therapeutic potential of antisense ODNs, however, has been questioned because of confounding issues regarding the specificity of this approach in biological systems.37-40 There are several lines of evidence confirming the specificity of the c-myc antisense effects observed in the present study: (1) the inhibition of growth and type I collagen synthesis was observed only with antisense ODNs, but not with sense, scrambled, or mismatched sequences; (2) we have previously shown that antisense ODNs targeting translation initiation codon of human c-myc mRNA downregulate c-myc expression, whereas sense ODNs did not;41 (3) other antisense sequences against the c-myc proto-oncogene exert similar inhibitory effects (unpublished data), which excludes “non-antisense” mechanisms (eg, aptamer)41; (4) c-myc antisense ODNs in a dose range producing growth and type I collagen inhibition did not cause a significant change in the metabolic state of VSMCs; and (5) sense oligomers did not affect neointimal formation, whereas antisense ODNs modified various morphometric parameters.

This study is the first to demonstrate that a single transcatheter administration into the coronary arterial wall was effective in reducing neointimal formation. ODNs were delivered immediately following balloon denudation, thereby eliminating the need for the additional instrumentation. An intramural administration lasting less than 30 seconds makes a transcatheter approach particularly attractive for the application in the coronary vasculature. A single endoluminal injection resulted in the localization of ODNs at the site of coronary denudation for at least 3 days. The remaining
TABLE 3. Histological Injury Score

<table>
<thead>
<tr>
<th>Grade</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Neointima present</td>
</tr>
<tr>
<td>Intima</td>
<td>Punctuated focal breaks</td>
</tr>
<tr>
<td>Media</td>
<td>Intact</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Neointima present</td>
</tr>
<tr>
<td>Intima</td>
<td>Fragmented with a distinct gap</td>
</tr>
<tr>
<td>Media</td>
<td>Disrupted, partially replaced by neointima (inner third)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Neointima present</td>
</tr>
<tr>
<td>Intima</td>
<td>Fragmented with a distinct gap</td>
</tr>
<tr>
<td>Media</td>
<td>Disrupted, partially replaced by neointima (inner two thirds)</td>
</tr>
<tr>
<td>Grade 4</td>
<td>Neointima present</td>
</tr>
<tr>
<td>Intima</td>
<td>Fragmented with a distinct gap</td>
</tr>
<tr>
<td>Media</td>
<td>Disrupted, neointima extends to adventitia</td>
</tr>
</tbody>
</table>

IEL indicates internal elastic lamina.

ODNs lost into the circulation were rapidly cleared from the plasma, transiently accumulating in the liver and kidney, which is consistent with previous studies examining the biodistribution of ODNs.42-44 Of note, ODN concentration was increased in the coronary arterial wall 3 days after transcatheter administration. This may be due to differences in ODN delivery, which depends on the conditions of intramural injection (eg, balloon-to-artery ratio). However, redistribution of ODNs from repository organs to denuded vessels cannot be entirely excluded since ODN concentrations in the liver and kidney were declining at the same time. Additional studies are necessary to determine the exact localization of ODNs in the denuded coronary arterial wall as well as their intactness.

Study Limitations and Future Directions

Our results suggest that the reduction in neointimal formation in nonatherosclerotic coronary arteries was likely due to inhibition of cell proliferation and extracellular matrix synthesis since both were downregulated by c-myc antisense in vitro. Notwithstanding the above findings, the ability of antisense ODNs to inhibit neointima in lesions with underlying atherosclerosis remains to be determined. The presence of phenotypically modulated VSMCs and macrophages in the atherosclerotic plaque before balloon denudation may modify vessel wall response to injury. Thus, the present study provides the rationale to discern the effects of c-myc antisense ODNs on neointima in an atherogenic environment, which may render information regarding the pathomechanism of restenosis in patients. In addition, differences in the vessel wall content (eg, fibrous elements and calcifications) may affect the biodistribution of oligomers within target lesions. In the present study, a small volume of the injectate was delivered using low pressure to minimize vascular injury related to intramu-

Fig 5. Maximal neointimal area plotted as a linear function of histological injury score for the control and c-myc antisense-treated groups. The comparison of regression lines demonstrated significant difference by slopes ($P=.001$).

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Fig 6. Photomicrographs of porcine coronary arteries (Verhoff–van Gieson stain) at 1 month after balloon injury and sense (top) or c-myc antisense oligodeoxynucleotide delivery (bottom). Despite similar severity of vessel injury with disruption of the internal elastic lamina and media (grade 4), neointimal formation is decreased after antisense oligodeoxynucleotides (1 mg). Original magnification ×15.
ral injections.\textsuperscript{45} Alternative modalities based on either new device technologies\textsuperscript{46,47} or enhanced cellular uptake of ODNs\textsuperscript{13} are being actively pursued by many investigators to optimize local drug delivery at the time of coronary angioplasty.

**Conclusions**

(1) Single transcatheter administration allowed endoluminal delivery of oligomers to the site of coronary arterial injury. (2) c-myc antisense oligomers reduced the formation of neointima in denuded coronary arteries, implying a therapeutic potential of this approach. (3) It is postulated that the c-myc proto-oncogene is involved in the process of vascular remodeling in response to injury, modulating smooth muscle cell proliferation and matrix synthesis.

**Acknowledgments**

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