SAPHENOUS VEIN GRAFT PROTECTION: EFFECTS OF *c-myc* ANTISENSE

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Objective: Saphenous vein grafting is associated with extensive medial remodeling, characterized by cellular proliferation, loss of smooth muscle cells, and an inflammatory response. In this study, we examined whether unfavorable responses to vein grafting could be modified by the intraoperative application of *c-myc* antisense oligomers. **Methods:** The intragraft cell proliferation, macrophage infiltration, and medial preservation were examined in a porcine model in the control and antisense-treated groups ($n = 36$). **Results:** Saphenous veins showed transmural distribution of oligomers within 30 minutes of the ex vivo incubation. A concentration-dependent inhibition of cell proliferation in the media of saphenous grafts was noted 3 days later (0 to 200 $\mu\text{mol/L}$, $p = 0.005$). The growth inhibition was sequence-specific, because control oligomers produced only insignificant effects (20 $\mu\text{mol/L}$). Vascular effects of *c-myc* antisense were associated with a significant attenuation of macrophage infiltration in saphenous grafts. A concentration-dependent decrease in tissue edema ($p = 0.0005$) and the attenuated loss of smooth muscle cells ($p = 0.002$) were noted in the media of the arterialized venous conduits after *c-myc* antisense. **Conclusions:** Direct application of synthetic DNA to harvested saphenous veins resulted in a rapid transmural distribution. The inhibition of the intragraft cell proliferation in vivo after *c-myc* antisense was sequence dependent. Decrease in vein graft injury resulted in an attenuated inflammatory response and better medial preservation. These findings provide a rationale for assessment of the long-term effects of vein graft protection with *c-myc* antisense. (J Thorac Cardiovasc Surg 1998;115:152-61)

Bypass grafting with autologous saphenous vein has become the cornerstone of therapy for obstructive coronary artery disease since its introduction by Favaloro in 1969.¹ Clinical outcomes of this procedure, however, are marred by a progressive loss of saphenous vein graft (SVG) patency, which is particularly accelerated at later years.^{2,3} The preexisting changes in saphenous vein may have an impact on the fate of SVGs.⁴ More important, how-

ever, vascular injury is commonly induced by grafting.⁵⁻⁷ These changes are accompanied by the rapid activation of immediate-early gene expression, including *c-fos* and *c-myc* protooncogenes, and contribute to cell proliferation.⁸ Subsequent events, including thrombosis, intimal formation, and even late graft atherosclerosis, can be viewed as the response to ubiquitous vascular injury that develops after vein arterialization.

Our previous observations indicated the presence of intragraft cell proliferation and suggested migration of myofibroblasts through the injured media to neointima.⁹ The accompanying changes resemble the process of wound healing and may result in unfavorable remodeling of the conduit.^{10,11} The intima, which often replaces damaged media, becomes the nidus for future atherosclerotic plaque.¹² On the basis of these observations, we examined the hypothesis that early changes in SVGs can be attenuated by the intraoperative application of an intervention aimed at controlling cell proliferation. In this study, we report concentration-dependent ef-

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fects of synthetic antisense DNA directed against the *c-myc* protooncogene in porcine SVGs. The results demonstrate reduction in cell proliferation and improved preservation of the media in SVGs. These data provide a rationale for the future examination of this strategy to improve the long-term patency of SVGs.

Methods

Animal preparation. A porcine model of SVG interposition in the common carotid artery was used.^{7,9,10} After medication with aspirin (650 mg orally) and cefazolin (1 gm intravenously), crossbred pigs ($n = 36$, 22 to 55 kg) were sedated with ketamine (20 mg/kg intramuscularly) and xylazine (4 mg/kg intramuscularly). The anesthesia was maintained with endotracheal halothane (0.75%) and oxygen. Lateral saphenous veins (6 to 10 cm) were isolated and then incubated in a saline solution bath (total volume 10 ml) with either no oligomers or various sequences, as described later. The carotid arteries were dissected free and heparin sodium (150 units/kg intravenously) was administered. After a 2 cm portion of the carotid artery was excised, the proximal end was anastomosed with a continuous suture, and a similar procedure was performed on the distal anastomosis. The incision was closed, and the animals were allowed to recover. Replicating cells in SVGs were labeled in vivo with an intravenous injection of 5-bromo-2'-deoxyuridine (30 mg/kg BrdU; Boehringer Mannheim Corp., Indianapolis, IN) 2 hours before the animals were euthanized.

The animals were euthanized with an intravenous overdose of Euthasol (Delmarva Laboratory, Midlothian, VA) containing pentobarbital sodium (1950 mg) and phenytoin sodium (250 mg) 3 days after the operation. All procedures were in accordance with institutional guidelines and in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Oligomer preparation and application. Phosphorothioate oligomers were synthesized by an automated deoxyribonucleic acid (DNA) synthesizer and purified by high-pressure liquid chromatography (Lynx Therapeutics, Hayward, CA). Table I depicts oligomer sequences that were used in this study. For vascular distribution studies, antisense oligomers were labeled with carboxyfluoresceins as described by the manufacturer (Applied Biosystems, Foster City, CA). Freshly excised saphenous veins were then incubated with oligomers for 2 seconds, 30 minutes, and 1 hour. For in vivo studies, individual saphenous veins were randomly assigned to different concentrations of antisense DNA (0, 2, 20, and 200 $\mu\text{mol/L}$) or different oligomer sequence (no oligomer, antisense, scrambled, or mismatched) as indicated in the text. After the 30-minute incubation period in the respective solution, saphenous veins were interposed in the carotid artery and the remaining bath solution was topically applied to the perigraft region.

Table I. *C-myc* mRNA and oligomer design used in the study

Name	Sequences (5' → 3')
Human <i>c-myc</i> mRNA	...CG AUG CCC CUC AAC GUU AG...
Porcine <i>c-myc</i> mRNA	...CG AUG CCC CUC AAC GUC AG...
Antisense	AAC GTT <u>GAG</u> <u>GGG</u> CAT
Scrambled	GAA CGG AGA CGG TTT
Mismatched	AAG CAT <u>ACG</u> <u>GGG</u> TGT

Human and porcine *c-myc* mRNA sequences are shown for comparison. The AUG region represents the first codon of the second exon of *c-myc* mRNA. The antisense sequence contains 15 nucleotides complementary to human *c-myc* mRNA and one nucleotide mismatch compared with porcine mRNA. Nucleotides shown in italics represent mismatches with porcine mRNA, whereas contiguous guanosines (4G motif) are underlined.

Tissue sampling, preservation, and processing. The distribution of fluorescent-labeled oligomers was examined in formalin-fixed (6 hours) and paraffin-embedded saphenous veins. Serial sections (4 μm thick) were removed from paraffin and covered with SlowFadeLight antifading reagent (Molecular Probes, Inc., Eugene, OR), and fluorescent-labeled oligomers were visualized under a Nikon Optiphot microscope (Nikon Inc., Instrument Group, Melville, NY). The conditions were optimized with a narrow-band filter (excitation 485 ± 10 nm) that eliminated the autofluorescence in the control saphenous veins.

In the remaining experiments, carotid arteries were exposed at the time of SVG harvesting. Distal ends were opened to demonstrate pulsatile blood flow and ensure graft patency in situ. Occluded vessels were excluded from further analysis (0, 3, 2, and 4 in 0, 2, 20, and 200 μmol groups, respectively, not significant), whereas patent vessels were excised with surrounding tissues and immersed in Histo-Choice fixative (Amresco, Solon, OH) for at least 5 hours. They were sectioned (5 mm blocks), processed in a TissueTek vacuum infiltration processor (Miles Scientific, Naperville, IL), and embedded in paraffin. The sections (5 μm thick) from each block were stained with Verhoeff's stain to determine the overall vessel structure. Multiple sections from at least three blocks representing the body of the graft were selected for this study. The n values reported in the Results section represent the number of vessels.

Immunohistochemistry. The Vectastain Elite ABC system (Vector Laboratories, Inc., Burlingame, CA) was used as previously described.⁹ The following primary antibodies were used: a monoclonal mouse antibody recognizing BrdU (1:200, Novocastra Laboratory, Ltd., Newcastle Upon Tyne, UK), a monoclonal mouse DE-R-11 antibody recognizing intermediate filament desmin (1:50; Novocastra); a monoclonal mouse antiporcine macrophage immunoglobulin G_{2b} antibody (1:10, ATCC HB 142.1; American Type Culture Collection, Rockville, MD). Afterward, slides were incubated with biotinylated secondary horse antimouse immunoglobulin G (1:2000; Vector Laboratories) for 1 hour. They were then stained with diaminobenzidine tetrahydrochloride substrate kit (Vector Laboratories) followed by counterstain with Gill's hematoxylin (Sigma Diagnostics, St. Louis, MO). Positive control preparations for desmin included sections of

Fig. 1. Time-dependent vascular distribution of fluorescent oligomers. Human saphenous vein segments were incubated ex vivo with *c-myc* antisense DNA (200 $\mu\text{mol/L}$) and examined by fluorescent microscopy. **A**, At 2 seconds, fluorescence is visible only along luminal and adventitial surfaces, whereas the media (*m*) is devoid of oligomers. Note the absence of autofluorescence in the media. **B**, At 30 minutes, transmural distribution of oligomers is associated with nuclear localization of fluorescence. **C**, At 1 hour, the media (*m*) is further saturated with labeled oligomers. Experiments were repeated three times, yielding similar results. (Magnification $\times 25$.)

porcine coronary arteries and normal human saphenous veins. For the antimacrophage antibody, positive control preparations consisted of lung alveolar macrophages. Negative control procedures were carried out with non-immune serum instead of primary antibody.

Data analysis. To examine concentration- or sequence-dependent effects of oligomers on cell proliferation, cells showing BrdU in at least three different sections from each graft were counted without the knowledge of random assignment. To avoid selection bias, quantitative measurements were carried out in the entire media under the same magnification; mean values were then calculated. Histologic markers of vein graft injury (vessel morphometry, desmin immunostaining, macrophage count) were analyzed in serial sections adjacent to those with the highest number of BrdU-labeled cells in each vessel to account for the focal changes after vein grafting. To quantify medial changes (vessel morphometry, desmin immunostaining), the sections were digitized with a Sony DXC-750MD video camera (Sony Corporation, Tokyo, Japan) attached to a Nikon Optiphot-2 microscope. To obtain medial areas, sections stained with Verhoeff's stain were then processed with a video analysis software package (Media Cybernetics, Silver Spring, MD). To quantify the loss of medial smooth muscle (SM) cells after grafting, digitized images of desmin immunostaining were analyzed with the Image-Pro software (Media Cybernetics). From each ves-

sel, a microscopic field exhibiting the worst medial damage was selected. The area occupied by desmin was then automatically quantified and expressed as the percentage of media.

Numeric data are presented as mean $\pm$ standard error. To determine whether the effects of *c-myc* antisense DNA were concentration dependent, statistical significance was calculated with linear regression analysis. Furthermore, analysis of variance was carried out to examine the overall differences in multigroup comparisons. If a significant *p* value was present, Bonferroni correction was used to identify specific intergroup differences. A *p* value of less than 0.05 was considered statistically significant.

Results

Distribution of antisense DNA in saphenous vein conduits. To examine the distribution and time course of oligomer uptake in saphenous veins, freshly harvested segments were incubated ex vivo with fluorescent-labeled antisense DNA (200 $\mu\text{mol/L}$) for 2 seconds, 30 minutes, and 1 hour. As shown in Fig. 1, transmural distribution of oligomers was apparent after 30 minutes. Nuclear localization of the label pointed to the intracellular uptake of antisense DNA. A similar transmural distribution of

Fig. 2. Cell proliferation in porcine SVGs. Harvested saphenous veins were treated with no oligomers (A) or with *c-myc* antisense DNA at concentrations of 2 $\mu\text{mol/L}$ (B), 20 $\mu\text{mol/L}$ (C), or 200 $\mu\text{mol/L}$ (D). Proliferating cells were labeled in vivo with BrdU at 3 days after vein interposition into the carotid arteries. BrdU-labeled cells (brown nuclei) are decreased in the media (m) of SVGs treated with 20 $\mu\text{mol/L}$ and 200 $\mu\text{mol/L}$ of *c-myc* antisense DNA. Arrows point to the border between the media (m) and adventitia. Labeled cells outside the media represent proliferating cells in the adventitia and perivascular wound. (Magnification $\times 150$.)

oligomers was also evident by direct perivascular application of oligomers to the vessels in situ (not shown), which suggested that the pattern of oligomer uptake was not related to tissue damage associated with vein isolation. On the basis of these results, the 30-minute incubation period was selected for the remaining in vivo experiments.

Antiproliferative effect of antisense DNA in SVGs.

A dose response regarding the inhibition of cell proliferation was established in SVGs in vivo. Medial cell proliferation was compared after the application of no oligomers ($n = 6$) or *c-myc* antisense DNA at concentrations of 2 $\mu\text{mol/L}$ ($n = 5$), 20 $\mu\text{mol/L}$ ($n = 6$), and 200 $\mu\text{mol/L}$ ($n = 6$). Fig. 2 illustrates the intragraft cell proliferation (BrdU

labeling), which was decreased in a dose-dependent manner by antisense DNA ($p = 0.005$). As summarized in Fig. 3, proliferative responses were significantly reduced at 20 $\mu\text{mol/L}$ (decreased by 60%) and 200 $\mu\text{mol/L}$ concentrations (decreased by 82%) at 3 days after the treatment.

To examine whether the antiproliferative actions of antisense DNA are sequence dependent, as opposed to the effects related to phosphorothioate backbone (sequence independent) or the presence of four contiguous guanosine residues (4G motif), we also compared the inhibition of cell proliferation in vivo with two different control sequences. As shown in Table II, antisense produced the most pronounced inhibition of the intragraft cell prolifer-

Fig. 3. Bar graph illustrating reduction in medial cell proliferation after *c-myc* antisense DNA in SVGs. Results are presented as mean $\pm$ standard error, with *p* values referring to comparisons between treatment and control (no oligomers) groups.

ation ($p = 0.02$), whereas scrambled oligomers or mismatched sequences (4G motif) were devoid of significant effects. It is noteworthy, however, that control oligomers with 4G motif produced some decrease in cell proliferation in vivo at equimolar concentrations.

Reduction of inflammatory response after antisense DNA treatment. The changes associated with saphenous vein grafting include an inflammatory response, which may increase graft injury. Accordingly, we compared macrophage content in the untreated and the antisense-treated SVGs 3 days after the operation (Fig. 4). Total macrophage content was significantly reduced from 587 ± 153 cells/graft in saphenous veins incubated without oligomers ($n = 6$) to 159 ± 52 ($20 \mu\text{mol/L}$, $n = 6$, $p = 0.03$) and 104 ± 26 cells/graft ($200 \mu\text{mol/L}$, $n = 6$, $p = 0.02$) after *c-myc* antisense DNA treatment. As shown in Fig. 5, these differences were due mainly to the decrease in infiltrating macrophages, whereas the reduction in adhering macrophages did not reach statistical significance.

Medial preservation after antisense DNA. As shown in Fig. 6, the untreated SVGs typically demonstrated several microscopic characteristics consistent with medial injury (focal cell infiltrates, cellular debris, and intramural hematoma or edema). This contrasted with the antisense-treated grafts, which exhibited better-preserved media (Fig. 6). To further examine the vasculoprotective effects of antisense DNA, we quantified the markers of medial

Table II. Sequence-dependent effects of *c-myc* antisense oligomers on cell proliferation in vivo

Sequence	n	Proliferation Δ (%)	p
Antisense	8	-65 ± 6	0.02
Scrambled	8	$+6 \pm 23$	NS
Mismatched	8	-29 ± 11	NS

Oligomers were applied in equimolar concentrations ($20 \mu\text{mol/L}$). Cell proliferation was assessed by the number of BrdU-labeled cells in the media of the graft at 3 days after the treatment (random allocation). Changes (Δ) were calculated as a percentage in comparison with the control group ($n = 8$, no oligomers). Negative values represent a reduction in cell proliferation; positive value refers to an increase. Results are presented as mean $\pm$ standard error, *p* value refers to intergroup comparisons with control group (analysis of variance followed by Bonferroni's correction).

preservation. A concentration-dependent (0 , $20 \mu\text{mol/L}$, $200 \mu\text{mol/L}$; $p = 0.0005$) reduction in medial edema was indicated by the decrease in medial areas after the treatment (Fig. 7). To confirm that these effects were associated with the improved SM cell retention, the distribution of desmin (a marker of SM cells) was also analyzed. As illustrated in Fig. 8, the untreated vein grafts typically showed focal loss of SM cells, whereas the antisense-treated veins showed better preservation of desmin. These effects were concentration dependent (0 , $20 \mu\text{mol/L}$, $200 \mu\text{mol/L}$; $p = 0.002$). The retention of desmin in the media is summarized in Fig. 9.

Discussion

Cell proliferation is recognized as a typical response of vascular tissues to injury. This phenomenon may contribute to undesirable sequelae, such as SVG degeneration after revascularization procedures.^{9,10} This study demonstrated rapid development of cell proliferation and medial injury in the arterialized SVGs. The intraoperative incubation of saphenous veins with antisense DNA against *c-myc* conferred significant vasculoprotective effects, reflected by the reduction in cell proliferation and the decrease in several markers of medial injury.

Sequence-dependent effects of "antisense" DNA. Vascular applications of antisense have been based on a premise that synthetic DNA complementary to messenger ribonucleic acid (mRNA) of a cell-cycle-regulating gene can block the proliferative reaction by means of DNA-mRNA hybridization.^{13,14} It should be emphasized, however, that antisense DNA oligomers are pleiotropic compounds that may exert their biologic effects through several mechanisms. Phosphorothioate backbone prolongs oligomer bioavailability, but it may also contribute to effects unrelated to oligomer-mRNA hybridiza-

Fig. 4. Inflammatory response in SVGs. **A**, Untreated saphenous veins exhibited focal accumulation of macrophages in the media (dark brown staining). **B**, Treatment with *c-myc* antisense oligomers (200 $\mu\text{mol/L}$) was associated with attenuated inflammatory response 3 days after the operation. Note the decrease in infiltrating macrophages in the media of the graft, whereas adhering macrophages at the luminal side were less affected. Arrows point to the border between the media and adventitia. Labeled cells outside the media represent macrophages in the adventitia and perivascular wound. (Magnification $\times 150$.)

tion.¹⁵ It is important to recognize that even some sequence-dependent phenomena are related to interactions with growth factors (e.g., basic fibroblast growth factor), thereby augmenting antiproliferative effects.¹⁶ This study demonstrated sequence-dependent reduction in medial cell proliferation in SVGs, whereas scrambled phosphorothioate sequence exhibited no effects in vivo (Table II). These findings underscore the dichotomy between in vitro and in vivo systems, with the former more likely to elicit sequence independent effects.¹⁵⁻¹⁷ We also tested a mismatched sequence containing 4G motif, notorious for several nonantisense effects in cell culture.^{17, 18} When different oligomers were compared in vivo with approximately the median effective concentration, control sequence with 4G motif did not demonstrate a significant decrease in cell proliferation. Accordingly, the antiproliferative effects of antisense DNA in vivo cannot be attributed solely to the presence of contiguous guanosine residues. However, the present findings do not exclude the possibility that 4G motif confers additive antiproliferative effects at higher concentrations.

The intracellular accumulation of intact antisense oligomers is associated with downregulation of *c-myc* expression in cells derived from saphenous veins.¹⁴ Nonetheless, because the *c-myc* gene represents a common final pathway in the growth regulation of vascular cells, a wide range of other growth

Fig. 5. Bar graph illustrating changes in inflammatory response in SVGs after treatment with *c-myc* antisense oligomers. Total macrophage content and infiltrating macrophages in the media were significantly reduced in treated groups. The number of adhering macrophages, although reduced, did not reach statistical significance. Results are presented as mean $\pm$ standard error, with *p* values referring to comparisons between control (oligomers) and individual treatment groups (20 $\mu\text{mol/L}$ and 200 $\mu\text{mol/L}$).

inhibitors may reduce its expression without direct interactions with target mRNA.¹⁹ Accordingly, downregulation of the target mRNA or protein is not sufficient to discern between overlapping antisense (oligomer-mRNA hybridization) and nonantisense (e.g., growth-factor inhibition) mechanisms.

Fig. 6. Photomicrographs showing the media of SVGs (Verhoeff's stain). **A**, Untreated grafts demonstrated medial edema, focal necrosis, and, frequently, adhering layer of thrombus. Note disorganized cells in the media. **B**, Treatment with *c-myc* antisense oligomers (200 $\mu\text{mol/L}$) was associated with reduction of medial edema and overall better preservation of SM cells in the graft. Note circumferential alignment of SM cells in the media. Arrows point to the border between the media (*m*) and adventitia. (Magnification $\times 150$.)

Fig. 7. Bar graph depicting changes in medial areas of SVGs after *c-myc* antisense oligomers. Medial areas are significantly smaller in the treated group (200 $\mu\text{mol/L}$) as a result of marked reduction in tissue edema and inhibition of inflammatory response. Results are presented as mean $\pm$ standard error, with *p* values referring to comparisons between treatment and control (no oligomers) groups.

Taking into consideration these observations, we prefer to describe the effects of *c-myc* antisense oligomers as sequence dependent, rather than categorically describe them as purely antisense.

The role of media in vascular response to injury.

The observed changes in the SVGs have underscored a rapid development of medial injury, con-

sistent with previous experimental and clinical findings.^{6, 20} The activation of vascular fibroblasts with their differentiation to myofibroblasts develops after severe medial injury in coronary arteries.^{21, 22} Although the changes in SVGs differ from the arterial response to injury, some aspects of vascular repair are common.²³ Both perivascular and medial fibroblasts contribute to the repair of porcine arterialized saphenous veins translocating to the neointima.⁹ Likewise, human saphenous veins in organ culture preferentially acquire neointima from the adventitia or the edges of broken media (repositories of vascular fibroblasts), but not from the intact media, despite stimulation with growth factors.²⁴ These observations underscore the notion that the preservation of the media in SVGs may have an impact on long-term patency.

The origin of medial damage after saphenous vein grafting is multifactorial. In addition to tissue injury during surgical harvesting, the activation of cells in situ and medial infiltration with blood-borne inflammatory cells may induce local cytotoxic effects. The latter is particularly important because invading macrophages often perpetuate tissue damage.²⁵ A highly distinct mechanism leading to medial loss may be represented by apoptosis, which colocalizes in the regions of high proliferation and *c-myc* expression.²⁶ The results of this study demonstrate that *c-myc* antisense DNA attenuates several markers of tissue damage after SVG arterialization. The improvement in histologic preservation of medial SM cells, paralleled as it is by the

Fig. 8. SM cell preservation in SVG. **A**, Untreated grafts demonstrated focal loss of SM cell marker, desmin. Remaining medial SM cells (*brown cytoplasmic stain*) are interspersed with cell debris and nonmuscle cells. **B**, Marked retention of SM cells in the media was present in SVGs treated with *c-myc* antisense oligomers (200 $\mu\text{mol/L}$). Note the paucity of tissue edema and restored microarchitecture of the vein. Arrows point to the border between the media (*m*) and adventitia. (Magnification $\times 150$.)

decrease in macrophage infiltration, points to the possibility of vascular protection after arterialization.

Antisense DNA as a therapeutic agent. Antisense DNA sequences against several growth-regulatory genes have been envisioned for the prevention of coronary restenosis.^{27, 28} We previously showed the reduction in neointimal formation after antisense oligomers against the *c-myc* in a porcine model of coronary injury.²⁸ This approach, however, is hindered by a low efficiency of transcatheter delivery ($<1\%$). In contrast, direct ex vivo application of antisense DNA to the SVG results in the uniform distribution of oligomers after 30 minutes of incubation, involving not only the adventitia but also the entire media. This is particularly important for SVG, because the events in the media may contribute to late graft failure. Because the treatment of saphenous veins occurs ex vivo, the risk of potential side-effects in regions remote from the site of application of antisense DNA is probably minimized. Furthermore, it is noteworthy that identical antisense DNA has demonstrated no significant adverse effects even after the intracoronary administration in patients after coronary angioplasty.²⁹ The question of whether the short-term effects of *c-myc* antisense, as demonstrated in the current study, will translate to a long-term benefit regarding remodeling of the arterialized grafts requires separate studies. It should be underscored, however, that medial injury appears to occur early, so SVG protection may not necessarily require prolonged or repeated

Fig. 9. Bar graph showing retention of SM cells in SVGs after treatment with *c-myc* antisense oligomers. In areas with maximal histologic medial injury, desmin distribution was quantified. In the antisense-treated group (200 $\mu\text{mol/L}$), SM retention was significantly improved compared with untreated grafts. Results are presented as mean $\pm$ standard error, with *p* values referring to comparisons between control (no oligomers) and individual treatment groups.

drug administration. Alternative approaches may include attempts to prolong the bioavailability and cellular uptake of oligomers by complexing them with liposomes.³⁰

In conclusion, antisense DNA against the *c-myc* protooncogene can easily be delivered to harvested saphenous veins used for arterial revascularization, thus achieving the uniform distribution of oligomers

in the target vessel. This results in significant inhibition of cell proliferation and the reduction in medial damage that develops early in SVGs soon after grafting. The antiproliferative effects of antisense DNA are concentration and sequence dependent. These findings provide the basis for further studies of therapeutic potential of this approach to prevent long-term failure of SVGs.

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