The Role of Gene Therapy for Intimal Hyperplasia of Bypass Grafts

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Background—Proliferation of the intima is an early lesion of saphenous vein graft disease. Early patency rates of radial artery grafts are acceptable, but little is known about their risk of intimal hyperplasia.

Methods and Results—To develop a model of intimal hyperplasia, we incubated human saphenous veins, internal mammary arteries, and radial arteries (n=6, 8, and 10, respectively) in an organ culture with Rosewell Park Memorial Institute 1640 (30% serum) for 0, 4, 7, 10, and 14 days. Quantitative histological studies were performed, and the average intimal-to-medial (I/M) ratio was calculated for each incubation interval. After 10 and 14 days of culture, the I/M ratio increased in the saphenous veins (P=0.03, P=0.04 versus 0 day, respectively). No significant increase occurred in the I/M ratio in either the internal mammary or radial arteries. Next, the ability of adenoviral gene transfers to inhibit intimal hyperplasia in the saphenous veins was evaluated. Adenoviral-mediated gene transfer of nitric oxide synthase significantly reduced the I/M ratio at 14 days compared with vehicle (P=0.001) and virus (P=0.004) controls.

Conclusions—The human saphenous vein has a greater propensity for intimal hyperplasia than arterial grafts; the human radial artery behaves similarly to the internal mammary artery. In the future, gene therapy may augment nitric oxide synthase, limiting vein graft disease. (Circulation. 1999;100[suppl II]:II-392–II-396.)

Key Words: gene transfer ■ mammary arteries ■ nitric oxide ■ organ culture ■ radial artery ■ saphenous vein

Compared with saphenous vein (SV) grafts, internal mammary artery (IMA) grafts to the left anterior descending coronary artery improve the survival of patients with multivessel disease; this improved outcome correlates with better long-term patency.1 Recently, there has been renewed interest in the radial artery (RA) for coronary artery bypass in the hope that it will expand the range of arterial grafts for surgical revascularization.2 However, the long-term patency of RA conduits will not be known for nearly a decade, and little information is available about the biological behavior of these bypass conduits in regard to early thrombosis and the development of intimal hyperplasia and atherosclerosis.

The SV is the most commonly used conduit for coronary surgery, but despite improvements in methods of harvest, preservation, and early antithrombotic therapy, 12% to 27% of vein grafts become occluded in the first year, one-half of which are within the first month.2,3 The subsequent annual occlusive rate is 2% to 4%.4 More than 10% of patients who have coronary artery bypass with only the SV require reoperation within 10 to 12 years,5 and repeat operations represent 10% to 30% of coronary surgery in the United States.6

The purpose of the present study was to evaluate the potential of the human SV, IMA, and RA to generate intimal hyperplasia in an extended organ culture. After the propensity of the vein graft for hyperplasia was confirmed, the study evaluated the ability of adenoviral-mediated gene transfer of bovine endothelial nitric oxide synthase (eNOS) to inhibit this process.

Methods

The Institutional Review Board of the Mayo Foundation approved the use of human tissue. The procedures and handling of tissues exposed to recombinant adenovirus were approved by the Institutional Biosafety Committee of the Mayo Foundation in compliance with the Guidelines for Research Involving Recombinant DNA Molecules published by the National Institutes of Health (NIH Publication No. 59FR34496, amended 1995).

Unused segments of SV, IMA, and RA were obtained from 28 patients undergoing coronary artery bypass grafting; pairing of tissues was not possible. All the specimens had the usual surgical preparation in an attempt to duplicate a clinical correlate with the in vitro studies. Veins were harvested by sharp dissection and suture ligation of side branches via an extended lower extremity incision. RAs were harvested with sharp dissection and surgical clip ligation of side branches. Electrocautery was used for IMA dissection, with surgical clips for branches. At the completion of the anastomosis, the unused portions of bypass grafts were immediately immersed in cold (4°C) tissue culture medium (Rosewell Park Memorial Institute [RPMI] 1640 with HEPES buffer, 20 mmol/L, pH 7.4) containing

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After fixation, the specimens were immediately overlaid with 6 mL of polyester cloth) to sterile Sylgard resin (Dow Corning) with minute pins. The adventitial surface was then mounted (with the adventitial surface adjacent to P500 (Ad.CMVLacZ) for 1 hour at 37°C, as previously described. The lot of fetal bovine serum was used for the entire study.

The culture medium was aspirated and 37°C (Forma Scientific, Inc). The culture medium was aspirated for the entire specimen.

The number of positively stained cells was counted with standard light microscopy and 5-

Separate samples were snap-frozen (–70°C) in OCT compound, and 5-μm-thick sections were stained for eNOS and proliferating nuclear cell antigen, as previously described. The number of positively stained cells was counted with standard light microscopy and normalized to the product of the intimal and medial areas for the entire specimen.

Results are expressed as mean ± SEM. In all experiments, n is the number of patients from whom vessels were taken. Vessels incubated for each period were obtained from the same patient; analyses between incubation durations used the 2-way

Data Analysis

Results

Figure 1. I/M ratio (averaged over entire length of each specimen using image analysis system) for SV, IMA, and RA by days in organ culture. Note progressive increase in ratio in SV but not in IMA or RA. Values are mean ± SEM. n refers to number of vessels.

Intimal Hyperplasia With Organ Culture

Human SVs demonstrated progressively greater I/M ratios with extended culture, which were first significant after 10 days (Figure 1). The control I/M ratio of SV segments was 0.12 ± 0.04, and this increased to 0.28 ± 0.05 after 10 days (P < 0.03) and to 0.36 ± 0.11 after 14 days (P < 0.04). In contrast, after 14 days, the IMA I/M ratio increased to only 0.15 ± 0.04 (P = 0.20 versus control) and the RA I/M ratio, to 0.09 ± 0.01 (P = 0.15 versus control).

Previous studies suggested that the resulting intimal hyperplasia in extended organ culture is derived predominantly from the cut edges. The I/M ratios reported above represent the composite average of the entire specimen to eliminate sampling error. A subgroup analysis was performed of the I/M ratios within 1 optical field of the cut surface and the sample middle. For 7-day culture of SVs, the cut (0.25 ± 0.05) and middle (0.22 ± 0.10) I/M ratios were similar (P = 0.96); these ratios were not significantly different (0.43 ± 0.10 versus 0.67 ± 0.23; P = 0.65) at 14 days. Although some specimens had greater intimal hyperplasia at the cut surface, the subgroup analysis did not support the hypothesis of predominant growth in this region.

Adenoviral-Mediated Gene Transfer and Intimal Hyperplasia

Initial studies (n = 6) were performed at a viral titer of 10^{10} plaque-forming units per milliliter (pfu/mL). After 14 days of incubation, intimal hyperplasia was reduced in Ad.CMVeNOS veins compared with PBSA controls. However, a concomitant reduction occurred in the I/M ratio of...
The original study of extended organ culture used rabbit aortas to examine the endothelial response to injury. This model consistently produces neointimal overgrowth by smooth muscle cell migration, and it is devoid of fibroblast contamination. Intimal proliferation of human SV in organ culture is dependent on surgical preparation, production of vasoactive substances, and presence of growth factors. The advantages of this preparation include the manner in which human vessels can be maintained in controlled and reproducible conditions to analyze specific interventions. Human tissue can be manipulated ex vivo in a manner that would require animal studies for an in vivo setting.

However, as with all models, limitations exist. Although the human tissue underwent the usual surgical manipulation, the preparation does not duplicate the clinical setting because the system is without immunologic, cytokine, and hemodynamic influences. Despite these shortcomings, the model seems to be a good alternative to in vivo studies.

The specimens harvested in the present study correlate well with previously published morphometric data. The mean intimal and medial thicknesses of SV were similar to the 100 consecutive veins examined by Varty et al. The greater wall thickness in RAs than in IMAs correlated well with the results of van Son et al., except that the RA specimens in our series showed less of a differential.

RA Grafts
Reasonable early and midterm patency rates have been reported recently for RA grafts. Rates have ranged from 87.8% to 95.7% during the first 18 months. The reported 5-year patency rates range from 83% to 92%. Although these values are favorable compared with those for saphenous vein grafts, skepticism is still justified on the basis of the earlier history of RA grafts.

Although areas of preexisting disease have previously been noted, the average I/M ratio of RAs in our study was not different from that of other grafts. Organ culture of up to 14 days was not associated with significant intimal hyperplasia. This is in contrast to the behavior of SVs; the I/M ratio in veins nearly tripled during the same duration of incubation. The behavior of the RA in the current study, which was similar to that of the IMA, supports the continued, cautious, clinical use of this conduit.

Gene Therapy for SV Graft Disease
Neointimal hyperplasia is the ubiquitous response of the vasculature to injury; it results in a lesion that can be flow-limiting and consists of secretory vascular smooth muscle cells and extracellular matrix. The pathophysiology of neointimal hyperplasia after vascular injury has been studied extensively. In brief, endothelial injury and denudation permit platelet adhesion and aggregation during harvest or surgical exposure. The adherence of platelets to the endothelial surface is also likely due to decreased production of prostacyclin and nitric oxide concomitant with endothelial dysfunction. This is followed by stimulation of vascular smooth muscle cells to proliferate and to migrate to the intima. The smooth muscle cells undergo a phenotypic...
change, from contractile to secretory cells, and extracellular matrix accumulates. Platelet activation provokes the release of platelet-derived growth factor and basic fibroblast growth factor. Both factors, present in the fetal calf serum used for our culture preparation, stimulate the migration of vascular smooth muscle cells from the media into the intima and the synthesis of extracellular matrix, collagen, and proteoglycans.23

Our preparation of human SVs consistently produced intimal hyperplasia during 2 weeks of culture. The viral LacZ controls had a slightly less robust intimal hyperplasia than vehicle controls. Newman et al24 noted that intimal hyperplasia was markedly stimulated with an adenoviral vector encoding for LacZ in rabbit arteries. However, it seems that gene therapy elicits an immunologic response to either transgene expression or low-level viral protein expression on the cell membrane.25 In extended organ culture, no immunologic response was permitted; the culture media was acellular. Therefore, an intriguing suggestion from the present study is that highly promoted overexpression of a transgene, in and of itself, may tend to inhibit intimal hyperplasia.

Augmented nitric oxide levels inhibit the intimal hyperplasia of grafts. Von der Leyen et al26 first demonstrated that gene transfer of eNOS inhibited intimal thickening in rat carotid arteries after angioplasty. Immunohistochemical examination demonstrated increased expression of eNOS with adenoviral-mediated gene transfer; this is similar to findings we have reported previously in animal and human tissue.8,9,27 That eNOS overexpression inhibited SV hyperplasia is not entirely unexpected. Soyombo et al16 demonstrated that the 8-bromocyclic guanosine monophosphate analogue inhibited SV neointimal hyperplasia by 40%. What is remarkable is that the I/M ratio was similar for all groups at 7 days of incubation. The viral LacZ expression and function of a recombinant endothelial nitric oxide synthase-transduced human saphenous veins: gene therapy to augment nitric oxide production in bypass conduits. Circulation. 1997; 96(suppl II):II-173–II-178.


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