Local Gene Transduction of Cyclooxygenase-1 Increases Blood Flow in Injured Atherosclerotic Rabbit Arteries

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Background—Cyclooxygenase-1 (COX-1) is the rate-limiting component in the synthesis of prostacyclin (PGI2), an important vasodilator and antithrombotic molecule. In balloon-injured, atherosclerosis-free porcine arteries, COX-1 gene transduction increases PGI2 production, induces durable vasodilation, and reduces thrombus formation. We tested the effectiveness of COX-1 local gene transduction for the prevention of postangioplasty restenosis in atherosclerotic arteries in a hypercholesterolemic rabbit model.

Methods and Results—We injured 1 carotid artery in 43 Watanabe heritable hyperlipidemic rabbits and performed local gene transduction using a viral vector containing the COX-1 gene (AdCOX-1, n=22) or no genes (Adnull, n=21). Three days later, AdCOX-1–treated arteries stimulated with arachidonic acid produced 100% more PGI2 (P<0.01), 400% more prostaglandin E2 (PGE2) (P<0.01), 400% more prostaglandin E1 (PGE1) (P<0.01), and 250% more cAMP (P<0.05) than Adnull-treated arteries. Twenty-eight days after treatment, Doppler sonography showed that blood flow velocity was preserved in AdCOX-1–treated arteries (ratio 0.92, injured compared with contralateral uninjured carotid artery) but reduced in Adnull-treated arteries (ratio 0.39), suggesting that AdCOX-1 prevented restenosis after injury. COX-1–transduced arteries also showed 80% greater lumen area 28 days after injury (P<0.01).

Conclusions—The effectiveness of COX-1 in preventing restenosis and preserving normal blood flow 28 days after injury results from increased lumen area caused by durable vasodilation. COX-1 efficacy correlates with an early increase in the production of PGI2, PGE2, PGE1 (known to cause vasodilation), and cAMP. These results demonstrate for the first time that COX-1 gene transduction is an effective treatment for the prevention of postangioplasty restenosis of atherosclerotic arteries under clinically relevant conditions. (Circulation. 2005;111:1833-1840.)

Key Words: blood flow ▪ gene therapy ▪ angioplasty ▪ thrombus ▪ prostaglandins

Although it has been almost 30 years since the introduction of angioplasty, restenosis after angioplasty-induced arterial injury remains a major problem. Initially, catheter-based interventions, such as balloon angioplasty and stenting, restore blood flow in obstructed arteries in more than 90% of patients. However, within 6 months, vasospasm, thrombosis, intimal hyperplasia, and constrictive remodeling cause clinically significant narrowing of arteries in 20% to 50% of treated patients.1 Even with the advent of drug-eluting stents (ie, sirolimus- and paclitaxel-coated stents), which can reduce restenosis rates to less than 10% in nondiabetic patients,2,3 restenosis remains an important clinical problem.

As a consequence, restenosis has become a target for local intravascular gene therapy. Various strategies have been used.4–12 One approach is to inhibit smooth muscle cell proliferation by using antisense ribozymes to suppress cell cycle regulators, such as transforming growth factor-β1,13 or to use decoy oligonucleotides for transcription factors, such as E2F.14–16 early growth response factor-1,17 or nuclear factor κB.18 A second approach uses genes that downregulate smooth muscle cell proliferation or migration; these include the genes for fortilin (translationally controlled tumor protein),19 inducible nitric oxide synthase,20 endothelial nitric oxide synthase,21 heme oxygenase-1,22,23 prostacyclin synthase,21,24,25 and cyclooxygenase-1 (COX-1).24,26 A third approach makes use of antithrombotic genes, such as the genes for tissue factor pathway inhibitor and hirudin.27–29 A fourth approach involves using cytotoxic genes, such as herpes simplex virus–thymidine kinase.30

Restenosis is the result of 2 concomitant processes: neointima formation and constrictive remodeling. The pathogenesis of intimal hyperplasia after vascular injury involves diverse signaling cascades that ultimately converge on vascular smooth muscle cells, stimulating their proliferation and migration.1,31 Thrombi that form at the site of injury contribute to reobstruction of the vessel through their bulk and through the release of growth and chemotactic factors that...
promote the migration and accumulation of intimal vascular smooth muscle cells.\(^2\)

Constrictive remodeling relates mainly to changes taking place in the adventitia. Significant constriction (negative remodeling) of the adventitia occurs as a result of massive proliferation of adventitial fibroblasts and their differentiation into myofibroblasts with \(\alpha\)-smooth muscle cell activity.\(^2\)

Few reports have examined the efficacy of gene transduction against postinjury restenosis in atherosclerotic animal models. In a previous study, we found that COX-1 gene transduction into vascular systems triggers vasoprotective actions, including increased prostacyclin (PGI\(_2\)) synthesis and vasodilation.\(^2\) However, this study did not examine the influence of COX-1 gene transduction on the vessel wall and did not attempt to correlate underlying changes, such as inhibition of neointimal proliferation, inhibition of constrictive remodeling, and increased production of PGI\(_2\), prostaglandins \(E_2\) (PGE\(_2\)), and PGE\(_1\) (all prostaglandins with major vasodilative actions), with preservation of flow, prevention of restenosis, vasodilatation, or inhibition of thrombus formation. Accordingly, we have examined regulation of intimal hyperplasia, constrictive remodeling, and preservation of flow by local COX-1 gene transduction in injured atherosclerotic carotid arteries of natively hypercholesterolemic rabbits.

**Methods**

**Construction of an Adenoviral Vector Expressing COX-1**

The adenoviral vector expressing COX-1 (AdCOX-1) contains a full-length human COX-1 cDNA (1.8 kbp in size) cloned into a replication-deficient adenoviral vector, as previously described.\(^2\)

This vector contains a cytomegalovirus early promoter, which drives the constitutive expression of COX-1. The Adnull virus, an identical adenoviral vector that contains no foreign genes, was constructed in the same manner. The AdCOX-1 and Adnull viruses were then purified. Viral particle concentrations and plaque-forming units were determined by both A260/280 ratio (Introgen Therapeutics, Inc) and plaque assays.

**Carotid Artery Balloon Injury, Local Transduction of COX-1, and Adnull Delivery**

Forty-three Watanabe heritable hypercholesterolemic (WHHL) rabbits\(^2\),\(^3\) of either sex (age range, 12 to 18 months) that were fed a normal rabbit chow were treated with either AdCOX-1 (\(n=22\)) or Adnull (\(n=21\)). Of these, 7 AdCOX-1–treated and 7 Adnull-treated rabbits were killed 3 days after the procedure, and another 4 rabbits from each group were killed 14 days after the procedure. The remaining AdCOX-1–treated (\(n=11\)) and Adnull-treated rabbits (\(n=10\)) were killed 28 days after treatment. Total plasma cholesterol levels at the time of treatment were similar in the rabbits treated with Adnull (403±10 mg/dL) and AdCOX-1 (406±73 mg/dL). Local COX-1 gene transduction to the balloon-injured carotid arteries of WHHL rabbits was performed according to the animal surgical procedure protocol approved by the animal committee at the University of Texas–Houston Health Science Center. As previously described,\(^2\) after anesthesia was induced, a No. 4 catheter introducer was placed in each rabbit’s right femoral artery. Heparin (150 U/kg) was administered to prevent arterial thrombosis during angioplasty. An angioplasty balloon catheter (2.5×20 mm; Baxter Healthcare Corp) was inserted into the femoral introducer and advanced to the right carotid artery by use of a guidewire under fluoroscopy. Balloon angioplasty was performed using 5 inflations to 8 atm for 30 seconds each. One minute of reperfusion was allowed between inflations. After angioplasty, the balloon was retracted 15 mm, and the proximal end of the damaged artery was ligated over the tip of the deflated angioplasty catheter with 2-0 silk over umbilical tape to prevent additional injury. All blood was removed from the damaged artery by repeated flushing with saline through the wire port of the angioplasty catheter. The distal portion of the damaged artery was then ligated. After the remaining saline was removed, 1×10^\(10\) PFU/mL of either AdCOX-1 or Adnull was gradually introduced into the isolated artery. The virus was allowed to incubate in the artery for 30 minutes, after which the virus was removed and the artery was rinsed with saline. On completion of surgery, all incisions were repaired, and the rabbits were allowed to recover.

**Carotid Blood Flow Velocity Measurements and Carotid Sampling**

Twenty-eight days after injury and gene transduction, 11 AdCOX-treated and 10 Adnull-treated rabbits were reanesthetized, the neck incision was reopened, and both carotids were gently isolated. Carotid blood flow velocity measurements were then taken with a pulsed Doppler flow probe.

Uninjured contralateral and virus-transduced carotid arteries were harvested from these 21 rabbits 28 days after arterial injury and gene delivery. After the rabbits were killed with sodium pentobarbital, a left thoracotomy was performed, and a catheter was introduced into the left subclavian artery and advanced into the aortic arch. The catheter was secured by ligation over the subclavian artery. After ligation of the distal ends of both carotid arteries, the aortic arch and both carotids were flushed with 200 mL normal saline. Five hundred milliliters of 10% formaldehyde was infused into both carotids at 100 mm Hg to complete the fixation. Two 20-mm arterial segments were harvested, 1 from the damaged area of the right carotid and 1 from the corresponding region of the left carotid, and placed in formaldehyde.

**PGI\(_2\), PGE\(_2\), and PGE\(_1\) Measurement**

Carotid arteries were extracted from 4 rabbits in each group (AdCOX and Adnull) 3 and 14 days after treatment. Twenty-millimeter arterial segments were obtained from both the injured, transduced site and the corresponding contralateral site. Each artery ring was then gently cut open to expose the lumen and further cut into 6 small pieces. Each piece was washed 3 times with DMEM (Gibco, Invitrogen Corp). Artery samples from each rabbit were incubated for 1 hour in DMEM supplied with 2% FBS and then for another hour in DMEM (2% FBS) with 20 μmol/L arachidonic acid (AA). Successive supernatants were collected for prostaglandin assays. The production of PGI\(_2\) (measured indirectly as 6-keto-PGF\(_1\alpha\), a stable metabolite of PGI\(_2\)), PGE\(_2\), and PGE\(_1\) in supernatants was determined by use of enzyme immunoassay (EIA) kits (PGI\(_1\), PGE\(_2\), Cayman Chemical; PGE\(_1\), Assay Designs). Supernatants were properly diluted with EIA buffer (provided by the manufacturers, above), and EIA assays were performed according to the manufacturer’s instructions. EIA plates were read by use of a microplate reader at 405 nm (Bio-Rad Laboratories Inc). Artery samples were further uniformly dried, weighed, and frozen in liquid nitrogen for cAMP measurements.

**cAMP Measurement**

Immediately after collection of supernatants for prostaglandin assays, artery pieces were frozen in liquid nitrogen and finely ground. The resulting powder was suspened in 5% trichloroacetic acid. After suspension and centrifugation of this solution, the resultant supernatant was mixed with ether. Once the ether separated from the hydroxysoluble layer, the ether was removed and the samples were vacuum-dried. They were further reconstituted in PBS and analyzed by ELISA (Cayman Chemical) for cAMP.

**Immunohistochemistry for COX-1 and COX-2**

Artery rings were obtained from AdCOX-1–treated (\(n=3\)) and Adnull-treated (\(n=3\)) rabbits 3 days after injury and transduction. Samples were taken from both the treated site and the uninjured corresponding contralateral site. Artery pieces were fixed in form-
aldehyde for 48 hours, then embedded in paraffin and thinly cut at 5-μm intervals. After inactivation of endogenous peroxidase, COX-1 immunohistochemistry staining was performed in a standard manner using as a primary antibody a specific anti-human COX-1 monoclonal antibody that was raised in mice and known to not cross-react with rabbit COX-1 (Oxford Biomedical Research). We initially confirmed the specificity of this COX-1 antibody for human COX-1 in vitro, in human-COX-1–transduced rabbit endothelial and smooth muscle cell cultures (data not shown). Diaminobenzidine (DAB) was used as a chromogenic substrate for color visualization. COX-2 staining was performed in a similar manner using a mouse monoclonal COX-2 antibody that is cross-reactive with rabbit COX-2 (Vector Laboratories). A COX-2–positive control was obtained after overnight incubation of artery pieces in DMEM (8% FBS) supplied with AA (20 μmol/L) for 1 hour. Supernatants were used for 6-keto-PGF₁α determinations as explained in Methods. B. After stimulation with AA and collection of supernatants, artery pieces were dried, weighed, and deposited in liquid nitrogen for determination of cAMP (expressed as fmol/mg of arterial tissue). C and D, Differential expression of PGE₂ and PGE₁ in presence or absence of AA, assessed by same process as used in 6-keto-PGF₁α measurements.

Figure 1. A, Differential expression of PGI₂ (measured indirectly as stable metabolite 6-keto-PGF₁α, and expressed as pg/mg of arterial tissue) in presence (AA+) and absence (AA−) of AA in AdCOX-1– and Adnull-treated injured carotid arteries and uninjured controls 3 days after injury. Carotid artery rings were taken from a 20-mm length of artery and gently cut into 4 to 6 small pieces. Artery pieces were washed 3 times with DMEM and incubated twice, first with DMEM (2% FBS) alone for 1 hour and then with DMEM (2% FBS) supplied with AA (20 μmol/L) for 1 hour. Supernatants were used for 6-keto-PGF₁α determinations as explained in Methods. B. After stimulation with AA and collection of supernatants, artery pieces were dried, weighed, and deposited in liquid nitrogen for determination of cAMP (expressed as fmol/mg of arterial tissue). C and D, Differential expression of PGE₂ and PGE₁ in presence or absence of AA, assessed by same process as used in 6-keto-PGF₁α measurements.

Computer-Assisted Quantitative Histomorphometric Evaluation of Intimal Hyperplasia, Lumen Area, and Restenosis

After pressure perfusion fixation with 10% buffered formaldehyde, the harvested carotid artery segments were gently filled with a latex solution to further prevent collapse of the vessel walls. The segments of injured and uninjured carotid arteries taken from each rabbit (AdCOX-1, n=11; Adnull, n=10) were placed in buffered formaldehyde, cut into rings 2 to 4 mm long, and embedded in paraffin. Between 7 and 9 arterial rings were obtained from each injured carotid segment and stained with Verhoeff–van Gieson elastic stain. Magnified images were captured by use of an Axioptot microscope (Carl Zeiss, Inc) and a digital camera (Leaf Systems Lumina). Images were processed with software from Optima Imaging Analysis Systems, version 6.5. Arterial stenosis at 28 days was computed as a percentage of the total arterial diameter as measured by quantitative histomorphometry. Histomorphometric evaluation was performed by a technician who was unaware of the treatment the rabbits had received.

Statistical Analyses

Comparisons of mean carotid blood flow velocity and histomorphometric parameters between the AdCOX-1–treated (n=11) and Adnull-treated (n=10) rabbits were performed by use of ANOVA between groups. Comparisons of mean blood flow velocities and mean production of PGI₂, PGE₁, and PGE₂ between various groups were performed by use of a Student t test. Results were considered
significant at a value of \( P<0.05 \). Descriptive data are presented as mean±SD.

**Results**

**PGI\(_2\), PGE\(_2\), PGE\(_1\), and cAMP Production**

In the carotid artery segments harvested from the 8 WHHL rabbits (4 AdCOX-1, 4 Adnull) killed on day 3, PGI\(_2\) production in COX-1–transduced artery pieces was 40% greater than in Adnull-treated pieces (\( P<0.05 \)). This difference in PGI\(_2\) expression increased to 100% with the addition of AA (\( P<0.05 \)) (Figure 1A). Similarly, cAMP levels were 250% higher in AA-treated, COX-1–transduced artery pieces than in AA-treated, Adnull-treated artery pieces (\( P<0.05 \)) (Figure 1B). The production of PGE\(_1\) was 26% higher in the COX-1–transduced artery pieces than in the Adnull-treated pieces, and this difference was augmented to 415% when AA was added to the AdCOX-1–treated samples (\( P<0.01 \)) (Figure 1C). Similarly, PGE\(_1\) synthesis was 400% higher in the AA-stimulated, AdCOX-1–treated artery pieces than in the Adnull-treated pieces (\( P<0.01 \)). In contrast, PGE\(_1\) production in the unstimulated AdCOX-1–treated artery pieces was comparable to that in the Adnull-treated pieces (Figure 1D). There was no difference between the Adnull and AdCOX-1 groups in PGI\(_2\), PGE\(_2\), and PGE\(_1\) production at 14 days (data not shown).

**COX-1 and COX-2 Immunostaining**

Three days after AdCOX-1 or Adnull transduction, artery rings were obtained from the treated and contralateral carotid arteries. After fixation in formaldehyde for 48 hours, artery pieces were embedded in paraffin and cut at 5-\( \mu \)m intervals. The COX-1–stained artery pieces showed specific human COX-1 expression in the human COX-1–transduced rabbit arteries and no expression in the Adnull-treated or uninjured rabbit arteries with a specific anti-human COX-1 antibody with no rabbit COX-1 cross-reactivity (Figure 2, A–D).

Our human COX-1 local gene transduction method involves some of both the residual endothelial cells and the smooth muscle cells. The pattern of staining shows a diffusion-gradient–dependent decrease of transduction, which is higher in the internal layers of the media and lower in the external media, clustering behind (micro)fractures of the lamina elastica interna. This is explained by the access pattern of the carrier virus, because the adenovirus can access smooth muscle cells only via lamina elastica interna breaks, which typically are highly variable in size and location after angioplasty (Figure 2, B–D).

A COX-2–positive control was obtained after overnight incubation in DMEM (8% FBS) in the presence of IL-1\( \beta \) (100 ng/mL) (Figure 3, A and B). There was uniform IL-1\( \beta \)–induced expression of rabbit COX-2 involving the entire thickness of the media, whereas there was no expression in either the human AdCOX-1–transduced arteries or the Adnull-transduced arteries (Figure 3, C–F). This shows that human COX-1 transduction does not induce immunohistochemically detectable expression of rabbit COX-2 and attenuates the concern of a marked biological response after injury and viral COX-1 gene transfer.

**Blood Flow Velocity and Restenosis**

Twenty-eight days after balloon injury, carotid blood flow velocity (a surrogate measure of carotid blood flow) was assessed with Doppler flow probes in both carotid arteries of 21 rabbits (AdCOX-1, \( n=11 \); Adnull, \( n=10 \)). Injured COX-1–transduced arteries showed blood flow velocities (1.87±1.34 kHz) comparable to those of the uninjured contralateral control carotid arteries (2.18±0.9 Hz) (Figure 4A). In contrast, blood flow velocity in the injured arteries treated with the control vector, Adnull (0.91±0.27 kHz), was markedly lower than in the uninjured contralateral vessels (2.18±0.09 kHz) (\( P<0.05 \)) (Figure 4A). In addition, the ratio of blood flow velocity in treated injured arteries to blood flow velocity in untreated contralateral control arteries (the T/C
ratio) was higher in the AdCOX-1–treated rabbits (0.92 ± 0.22) than in the Adnull-treated rabbits (0.39 ± 0.21), indicating almost complete preservation of flow in the COX-1–transduced arteries.

Histomorphometric Measurements

Direct measurement at 28 days showed that the lumen areas of the COX-1–transduced injured carotid arteries were 80% larger than those of the Adnull-treated injured arteries (*P* < 0.01) (Figure 5A). This finding is in concordance with the casual macroscopic observation of significant vasodilation of the COX-1–transduced injured arteries, the diameter of which was almost double that of either the uninjured arteries or the Adnull-treated injured arteries (Figure 6). However, quantitative histomorphometry at 28 days showed that the balloon-injured carotid arteries treated with AdCOX-1 had only a 15% reduction in intimal area compared with the Adnull-treated arteries (*P* = NS) (Figure 5B). Notably, there was a slight increase in the media area of the COX-1–transduced injured arteries (16%) relative to the Adnull-treated injured arteries (*P* = NS). However, the intima/media ratio was 28% lower in the COX-1–transduced injured arteries than in the Adnull-treated injured arteries (*P* = NS). In addition, carotid thrombi were observed at the site of injury in 2 arteries treated with Adnull and in none of the arteries treated with AdCOX-1 (*P* = NS).

With regard to the uninjured contralateral carotid arteries, extensive native carotid atherosclerosis was evident in these arteries in both groups. Average intimal cross-sectional areas

**Figure 4.** Blood flow velocity as measured by Doppler ultrasonography in injured and uninjured carotid artery segments, 28 days after treatment with AdCOX-1 or Adnull. A, Blood flow velocity in kilohertz and ratio of blood flow velocity in treated arteries to that in control arteries (T/C ratio). Control group includes measurements of blood flow velocity in both AdCOX-1 and Adnull rabbits at uninjured contralateral site, consistently left carotid artery in our design. B, Arterial stenosis measurements at 28 days, expressed as a percentage of total arterial diameter as measured by quantitative histomorphometry.
of the uninjured sites were 0.58±0.06 mm² in the AdCOX-1 group and 0.71±0.03 mm² in the Adnull group. The 2 groups also had similar degrees of vascular stenosis: 55±4% in the AdCOX-1 group and 45±6% in the Adnull group.

Discussion

We examined the ability of local COX-1 gene transduction to preserve blood flow and prevent restenosis in balloon-injured atherosclerotic arteries of WHHL rabbits. We found that local COX-1 gene transduction preserved blood flow velocity and reduced restenosis in the injured arteries. However, as shown by the minimal change in intimal area among the COX-1–transduced arteries, neointima formation was not significantly inhibited by COX-1. Instead, the main mechanism of stenosis reduction and flow preservation in the COX-1–transduced arteries was marked arterial dilation, as indicated by the increase in the lumen area. This dilation was associated with significant increases in PGI₂, PGE₂, PGE₁, and cAMP production 3 days after injury. However, later assessments (at 14 days) did not show any differences in PGI₂, PGE₂, or PGE₁ expression between AdCOX-1– and Adnull-treated injured carotid arteries. This finding was expected, given the use of a nonreplicating adenoviral vector, which limited the duration of COX-1 overexpression.

The lack of significant neointimal inhibition in the COX-1–transduced injured arteries suggests that reduction of blood flow does not depend only on neointimal formation but on other control mechanisms relating to inflammatory response to injury.⁴¹ This is especially true in a model such as the WHHL rabbit, in which significant endothelial dysfunction and high atherosclerotic burden are present.⁴²,⁴³

Thus, our results provide some important information about the timing and interdependency of the basic morphopathological mechanisms of (re)stenosis. Restenosis after arterial injury is attributable to the processes of neointima formation, constrictive remodeling, thrombosis, and late matrix deposition. The driving force behind the early response to injury, which may prime the vessel for severe stenosis, is often inflammation, triggering platelet deposition and vasoconstriction. Low flow states and altered (turbulent) shear stress are known to drive the remodeling/restenosis process in the first days after angioplasty.³⁸,³⁹ Local COX-1 gene transduction has little impact on neointima formation, but, by preserving blood flow, it significantly limits constrictive remodeling. This suggests that constrictive remodeling process starts early after injury, simultaneously with neointima formation. Furthermore, our model confirms that the mechanisms that underlie these 2 processes are driven at least partly by independent forces; constrictive remodeling is driven primarily by flow changes, whereas neointima formation results primarily from thrombotic and inflammatory processes.

In addition, our study confirms the view that the early stages of the remodeling of injured vessels, which occur in the first days after arterial injury, are particularly important in the restenosis process. The noted association between durable vasodilation and early, limited, short-term increases in PGI₂, PGE₂, PGE₁, and cAMP production implies that the subsequent limitation of the negative constrictive remodeling process is the result of early, durable changes in the media, given the pattern of gene transduction.

Even though residual endothelial cells have a higher transduction rate and a greater prostaglandin production rate, our model places greater importance on the vasodilative,
local, prostaglandin-mediated autocrine and paracrine mechanisms that converge on the smooth muscle cells in the media. Our findings suggest a dual role for smooth muscle cells, which by different and partly independent mechanisms become involved in the separate processes of neointima formation and constrictive remodeling. Because smooth muscle cells are the most common effector cells involved in both neointima formation and constrictive remodeling, they are an important therapeutic target. Prevention of arterial recoil and vasoconstriction and preservation of blood flow are considered the chief mechanisms by which stents deter unfavorable remodeling after angioplasty. Although stents are effective in this regard, bare metal stenting is followed by significant neointima formation, possibly even more than is associated with angioplasty alone. The finding that local COX-1 gene transduction does not worsen neointima formation in our model suggests that this technique has considerable potential value as an adjunct to stenting after angioplasty.

Although our study further supports the rationale of local COX-1 gene therapy for peripheral vascular disease, we caution that our observations were made in a limited time frame, so we cannot recommend local COX-1 gene transduction as a biomolecular alternative to stenting.

In our model, local COX-1 gene transduction may cause significant changes in the expression of other prostanoids and eicosanoids. Some of these, such as thromboxane A₂, have substantial vasoconstrictive and prothrombotic actions. We limited our focus to the production of PG₁₂, PG₂₂, and PG₂₃ because these are known to be strong vasodilators, and the specific morphological changes noted in our model suggest that vasodilation is the chief restenosis-preventing mechanism of COX-1 gene transduction. It is also important to note that the concomitant increase in PG₁₂, PG₂₂, and PG₂₃ was associated with high AMP production after stimulation with AA, highlighting the importance of intracellular second messengers in vasodilation and remodeling.

The significant augmentation of differential prostaglandin production in the AdCOX-1-transduced arteries after ex vivo stimulation with AA presents another restenosis prevention method with important translational potential. The combination of local COX-1 gene transduction and specific fatty acids, such as di-homo-γ-linolenic acid (DGLA), may promote a better prostaglandin production profile. In this setting, DGLA could facilitate local synthesis of much higher levels of PG₁₂, while maintaining PG₁₂ synthesis levels and reducing PG₂₃ synthesis to produce a higher PG₁₂/PGE₂ ratio. This process would take advantage of the strong vasodilative and antiinflammatory properties of PG₁₂ and reduce PG₂₃-driven proinflammatory activity. All of these changes in the expression profile would have the added benefit of concomitant mild anticoagulation, because DGLA is not a thromboxane A₂ generator (unlike AA) and because PGE₂ may even be a mild anticoagulant.

Choosing more effective gene transduction vectors than the one used in the present study may produce significantly better results and facilitate further evaluation of vascular COX-1 local gene transduction. Adeno-associated viruses, for example, are associated with less immune-inflammatory potential, more specific targeting of the smooth muscle cells, and longer expression of the transduced gene than are adenoviruses. Our technique of local vascular gene transduction could also be improved by increasing the virus concentration, which would allow the use of very low volumes and reduce the transduction time to as little as 2 minutes. Furthermore, because COX-1 transduction appears to induce durable vasodilation but does not allow neointimal proliferation, transferring COX-1 in combination with other genes could produce additive or even synergistic therapeutic effects. For example, transferring COX-1 and tissue factor pathway inhibitor together could induce vasodilation and inhibit both neointima and thrombus formation, greatly reducing the risk of future arterial occlusion.

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