Prostacyclin Synthase Gene Transfer Accelerates Reendothelialization and Inhibits Neointimal Formation in Rat Carotid Arteries After Balloon Injury

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Abstract—Prostacyclin (PGI₂), a metabolite of arachidonic acid, has the vasoprotective effects of vasodilation, anti-platelet aggregation, and inhibition of smooth muscle cell proliferation. We hypothesized that an overexpression of endogenous PGI₂ may accelerate the recovery from endothelial damage and inhibit neointimal formation in the injured artery. To test this hypothesis, we investigated in vivo transfer of the PGI₂ synthase (PCS) gene into balloon-injured rat carotid arteries by a nonviral lipotransfection method. Seven days after transfection, a significant regeneration of endothelium was observed in the arteries transfected with a plasmid carrying the rat PCS gene (pCMV-PCS), but little regeneration was seen in those with the control plasmid carrying the lacZ gene (pCMV-lacZ) (percent luminal circumference lined by newly regenerated endothelium: 87.1 ± 6.9% in pCMV-PCS-transfected vessels and 6.9 ± 0.2% in pCMV-lacZ vessels, P < 0.001). BrdU staining of arterial segments demonstrated a significantly lower incorporation in pCMV-PCS-transfected vessels (7.5 ± 0.3% positive nuclei in vessel cells) than in pCMV-lacZ (50.7 ± 9.6%, P < 0.01). Moreover, 2 weeks after transfection, the PCS gene transfer resulted in a significant inhibition of neointimal formation (88% reduction in ratio of intima/media areas), whereas medial area was similar among the groups. Arterial segments transfected with pCMV-PCS produced significantly higher levels of 6-keto-PGF₁α, the main metabolite of PGI₂, compared with the segments transfected with pCMV-lacZ (10.2 ± 0.55 and 2.1 ± 0.32 ng/mg tissue for pCMV-PCS and pCMV-lacZ, P < 0.001). In conclusion, this study demonstrated that an in vivo PCS gene transfer increased the production of PGI₂ and markedly inhibited neointimal formation with accelerated reendothelialization in rat carotid arteries after balloon injury. (Arterioscler Thromb Vasc Biol. 1999;19:727-733.)

Key Words: prostacyclin ■ prostacyclin synthase ■ restenosis ■ gene therapy ■ balloon injury

Vascular diseases are main life-threatening complexes in western countries. Arterial interventional therapy such as balloon angioplasty and stenting is presently a potential strategy against coronary artery disease. However, the efficacy of these techniques is limited by restenosis (ie, the recurrence of the treated lesions, in 20% to 50% of procedures). Although some pharmacological agents and devices have been shown to decrease the incidence of restenosis, the results are not sufficient and other novel therapies are required for the further decrease or elimination of restenosis.

Intensive studies of gene therapy against restenosis have been performed and have demonstrated its superior efficacy in some animal models. In these studies, the strategies for reducing restenosis may be categorized into 3 groups according to the processes: cytostatic strategies, which focus on the inhibition of cell cycle entry (eg, retinoblastoma protein RB, cyclin kinase inhibitor protein p21, tissue inhibitor of matrix metalloproteinase-1); cytotoxic strategies, which induce the death of cells that have entered the cell cycle (eg, thymidine kinase isozyme derived from herpes simplex virus, cytotoxic strategies, which affect cells at several stages in the progression of restenosis, such as antithrombosis, reendothelialization, and the inhibition of smooth muscle cell proliferation (eg, hirudin, c-type natriuretic peptide, endothelial nitric oxide synthase, prostaglandin H synthase-1, vascular endothelium growth factor). In a paracrine mechanism, a secreted molecule could exert effects on uninfected cells around infected cells, and more efficient inhibitory effects might thus be achieved. Moreover, a multifactorial molecule would be more effective for preventing restenosis than a simple SMC growth inhibition, because complicated mechanisms underlie the process of restenosis.

Prostacyclin (PGI₂), an arachidonic acid metabolite, binds to PGI₂ receptor, which induces the production of
cAMP.24 PGI₂ exerts its multiple effects mostly by the production of cAMP, such as vasodilation,26 anti-platelet aggregation,24–25 inhibition of smooth muscle proliferation,28 and the modulation of cholesterol turnover.29 PGI₂ may be involved in many events in the regulation of vascular tone and growth through these multiple effects. It was postulated that the barrier function of the endothelium is disrupted after balloon injury, and adhesion of platelets and release of their growth factors may induce proliferative changes and result in restenosis.29–34 The earlier that endothelial function could be recovered, the greater reduction of restenosis would be gained. To test our hypothesis that an overexpression of endogenous PGI₂ may accelerate the recovery from endothelial damage and inhibit neointimal formation in the injured artery, and to investigate the pathophysiological significance of endogenous PGI₂, we constructed a plasmid expressing PCS. In the present study, using a balloon-injured rat artery model, we examined whether the local delivery of the PCS gene could prevent proliferative changes without inducing systemic side effects.

Methods

Plasmid DNA Preparation

Based on the obtained sequence of the rat PCS gene,35 we designed the primers (forward primer, 5'-CATGTTCTGGCCGCGCT-3'; reverse primer, 5'-GGAAACAGCAGGGTGAGG-3'; underlined region indicates starting codon). Total cellular RNA was extracted from rat aorta by the modified method of Chomczinski and Sacchi,36 and subjected to reverse-transcription using a first-strand cDNA synthesis kit (Pharmacia Biotech). In each tube, oligo(dT)12 to 18 primer (Invitrogen Corporation) was used as a primer for first-strand cDNA synthesis. PCR was performed for 40 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute with a final 10 minute extension period. The PCR product was electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and extracted. The 1.6-kb rat PCS cDNA containing the open reading frame sequence (nucleotides 7 to 999) was amplified. PCR product was inserted into the pCR2.1 vector (Invitrogen) and subjected to restriction analysis. The 1.6-kb rat PCS cDNA was ligated into the pCMV-β-gal plasmid (Stratagene) at the self-ligation sites. The ligated plasmids were confirmed by a DNA sequence reader (LI-COR). The sequence of each plasmid was confirmed by the manufacturer’s protocol. The sequence of the rat PCS gene,35 we designed the primers (forward primer, 5'-CATGTTCTGGCCGCGCT-3'; reverse primer, 5'-GGAAACAGCAGGGTGAGG-3'; underlined region indicates starting codon). Total cellular RNA was extracted from rat aorta by the modified method of Chomczinski and Sacchi,36 and subjected to reverse-transcription using a first-strand cDNA synthesis kit (Pharmacia Biotech). In each tube, oligo(dT)12 to 18 primer (Invitrogen Corporation) was used as a primer for first-strand cDNA synthesis. PCR was performed for 40 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute with a final 10 minute extension period. The PCR product was electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and extracted. The 1.6-kb rat PCS cDNA containing the open reading frame sequence (nucleotides 7 to 999) was amplified. PCR product was inserted into the pCR2.1 vector (Invitrogen) and subjected to restriction analysis. The 1.6-kb rat PCS cDNA was ligated into the pCMV-β-gal plasmid (Stratagene) at the self-ligation sites. The ligated plasmids were confirmed by a DNA sequence reader (LI-COR).

Gene Transfer to Cultured Cells With pCMV-PCS

SMCs were explanted from rat aortas and cultured in DMEM supplemented with 10% FBS (GIBCO), 100 U/mL penicillin, and 100 μg/mL streptomycin. SMCs were grown in 60-mm dishes to 60% confluence and then transfected with 5 μg pCMV-PCS or pCMV-lacZ with 50 μL of a liposomal transfection reagent, Lipofectamin Plus (GIBCO). After transfection, the SMCs were incubated for 2 days. The medium was removed once and replaced with 1 mL of fresh medium containing 10 μM/L sodium arachidonate. At 60 minutes after addition of arachidonate, the medium was extracted. The 6-keto-PGF₁α and TxB₂ levels were measured. The concentrations of 6-keto-PGF₁α and TxB₂ were measured with chemiluminescence immunoassay kits (BioAssay Inc.).

Measurement of PCS Protein Levels

An immunoblotting analysis was performed using 0.05% sodium dodecyl sulfate-polyacrylamide gels with cell lysates.37 The proteins obtained from the SMCs were transferred to a polyvinyl difluoride membrane (Millipore Corp.). The membranes were blocked in skim milk, washed 3 times with Tris-buffered saline with 0.2% Tween-20, and incubated with anti-rat PCS antibody (1:500 in TBS/Tween-20; Cayman Chemical Co.) for 1 hour at room temperature. Membranes were washed 3 times with TBS/Tween-20, incubated with anti-rabbit immunoglobulin antibody (1:5000), washed again 3 times with TBS/Tween-20, and developed with enhanced chemiluminescence reagent (Amershams).

Evaluation of Transgene Efficiency in SMCs

Transfected SMCs were detected by LacZ gene expression using β-galactosidase staining (5 mMol/L K₄Fe(CN)₆, 5 mMol/L K₃Fe(CN)₆, 1 mg/mL 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside; Invitrogen) for 2 hours. Transduction efficiency was evaluated by the average percentage of positive cells in whole cultured SMCs in 5 to 6 eyefields.

Measurement of cAMP Levels

Cultured SMCs were mixed with 6% trichloroacetic acid. The solution was washed with water-saturated diethyl ether 3 times and lyophilized. cAMP levels at an appropriate dilution were measured by a enzyme immunoassay (EIA) kit (Amershams) following the manufacturer’s protocol.

PCS Gene Transfer In Vivo

Male Sprague-Dawley rats (Chubu Kagaku Shizai) weighing 300–350 g were maintained under a 12 hour light/12 hour dark cycle at 23°C and were fed standard laboratory chow (RC4, Oriental Yeast) and water ad libitum. Rats were anesthetized with 50 mg/kg sodium pentobarbital, administered intraperitoneally. The experimental procedure used to induce injury in the rat common carotid artery has been described in detail elsewhere.8 To prevent acute thrombosis during the procedure, an intravenous bolus of heparin (200 U/kg) was injected 5 minutes before vascular injury as Zoldhelyi et al22 reported. After cervical median incision, the distal right common artery and the region of bifurcation was exposed. A 2F balloon catheter (Baxter Healthcare) was inserted through the external carotid artery and advanced into the thoracic aorta. After inflation of the balloon, the artery was injured in position 3 times for 30 seconds each. Both ends of the injured artery were temporarily ligated, and 30 μg of pCMV-PCS, pCMV-lacZ, or PBS with 50 μL Lipofectamin reagent in a total volume of 200 μL was instilled into the lumen with a 26-gauge needle for 30 minutes. The catheter was carefully withdrawn and bleeding was prevented with glue used for thoracic surgery. After confirmation of carotid artery flow with a flow monitor, the wound was sutured. In a prior study, the extent of endothelial denudation was confirmed at 2 days after balloon injury by Evans blue staining. Systolic blood pressure was measured at 7 days by the tail-cuff method (BP98A, Softron). All experiments were performed in accordance with the guidelines of the International Committee for Thrombosis and Hemostasis and were approved by our Ethical Committee for Animal Experimentation.

PGI₂ and TxA₂ Production in Rat Carotid Artery

The carotid arteries treated with pCMV-lacZ and pCMV-PCS were dissected 7 days after injury (n = 6 in each group). The arteries were cut into 5-mm lengths, washed with PBS, and incubated in 1 mL of 0.1 mMol/L Tris-HCl containing 10 μM/L sodium arachidonate at 37°C for 30 minutes. The levels of 6-keto-PGF₁α, and TxB₂ in the media were measured with the enzyme immunoassay kits described above.

Localization of Plasmid in the Vessel Wall

Seven days after the instillation of pCMV-PCS and pCMV-lacZ, the animals were euthanized by the administration of an overdose of pentobarbital, and the arteries were perfusion-fixed in 2% glutaraldehyde and 0.2% paraformaldehyde. LacZ gene expression was detected by β-galactosidase staining for 24 hours, and counterstaining with eosin.

Proliferation Index of SMCs In Vivo

Proliferating SMCs were evaluated by the thymidine analogue BrdU labeling technique. The proliferation index was measured in the control, pCMV-lacZ, and pCMV-PCS transfected groups (n = 6 in each group) as Matsumo et al8 reported. BrdU was injected (50 mg/kg, SC) 1, 8, 16, and 24 hours before removal of the carotid...
artery at 7 days after vascular injury. BrdU-positive cells were stained with a murine monoclonal antibody (Amersham), followed by goat anti-mouse Ig antibodies conjugated to peroxidase and detected with DAB. Adjacent sections were also stained with hematoxylin for the detection of nonproliferating cells. The positive and negative nuclei were counted in the media and newly formed intima. The BrdU labeling index was calculated by the following formula: (positive nuclei stained by DAB) / (total nuclei stained by hematoxylin).

**Histological Assessment of Neointima and Vascular Smooth Muscle Layer**

Seven or 14 days after injury and transfer, rats were killed and carotid arteries were perfusion-fixed and harvested for paraffin embedding. Sections were stained with hematoxylin-eosin or elastica van Gieson. The extent of neointimal formation was quantified by computed planimetry of histologically stained sections. The cross-sectional areas of the blood vessel layers including the intimal area and medial area were quantified by using NIH Image (by Wayne Rasband, National Institutes of Health, USA). The intima/media (I/M) ratios were calculated from 10 to 12 individual cross-sections of each artery. The mean of these determinations was used to calculate the I/M cross-section ratios for each animal. For the evaluation of the recovery of endothelium, sections were incubated overnight with an anti-von Willebrand factor antisem conjugated to peroxidase (1:50; DAKO). After a wash in TBS, the sections were developed with DAB in Tris-HCl buffer, pH 7.6, for 10 minutes, and counterstained with hematoxylin. The reendothelialization index (reEI) was defined as the percentage of luminal circumference lined by newly regenerated endothelium in the inner lumen circumference. The mean of reEI was calculated from 10 to 12 cross-sections of each artery.

**Statistical Analysis**

ANOVA followed by the Scheffe’s post hoc test was used to determine significant differences in multiple comparison testing among the groups. Unpaired Student’s t test was used for comparisons between groups. Fisher’s exact probability test was used for the comparison of the incidence of thrombus occlusion in pCMV-lacZ- and pCMV-PCS-transfected vessels compared with the pCMV-lacZ-transfected controls (17.3 ± 0.5 and 7.5 ± 0.4 pmol/mg protein, n = 5 each, respectively).

**Results**

**Augmentation of Prostacyclin Production in Cultured SMCs by pCMV-PCS Transfer**

We initially determined whether the present plasmid vector system could transfer target genes to cultured rat aortic SMCs using pCMV-lacZ. The transgene efficiency in rat aorta SMCs confirmed by β-galactosidase staining was 21.5 ± 1.7% (n = 6). Then, we transfected pCMV-PCS to cultured SMCs to confirm the increment of PG12 synthesis. The baseline levels of 6-keto-PGF1α produced by pCMV-PCS- and pCMV-lacZ-transfected SMCs showed no significant difference from those of untransfected cells (Table). However, 60 minutes after the 10 μmol/L sodium arachidonate treatment, the production of 6-keto-PGF1α increased in both pCMV-PCS- and pCMV-lacZ-transfected SMCs, with a significantly higher production in the pCMV-PCS-transfected SMCs-2-fold that of the pCMV-lacZ-transfected cell (P < 0.05). In contrast, the TxB2 production in the SMCs did not change significantly, even after the treatment with arachidonic acid.

PCS protein expression was detected in SMCs by immunoblotting using a specific monoclonal antibody for rat PCS. In the pCMV-PCS-transfected cells, 2.1-fold higher PCS protein expression was observed compared with the pCMV-lacZ transfect SMCs (n = 5 each, P < 0.01, Figure 1). The intracellular cAMP concentration was measured by EIA. The cAMP level was 2.3 times higher in the pCMV-PCS-transfected SMCs compared with the pCMV-lacZ-transfected cells (17.3 ± 0.5 and 7.5 ± 0.4 pmol/mg protein, n = 5 each, respectively).

**Transduction of the Arterial Vessel Wall After pCMV-lacZ and pCMV-PCS**

The distribution of transgene expression was confirmed 7 days after lacZ gene transfer in balloon-injured rat carotid arteries. The pCMV-lacZ-transfected rat carotid arteries showed a diffuse transduction of the medial layer with a focal transduction of the adventitia (Figure 2). The transduction of SMCs in the vessel area amounted to 8.5 ± 0.7% (n = 6), as indicated by the blue coloration of the cell nuclei and cytosol after pCMV-lacZ transfer. The production of PG12 in the arterial vessels was evaluated by determining the 6-keto-PGF1α levels, 7 days after gene transfer. The 6-keto-PGF1α production levels were lower in control and pCMV-lacZ-transfected vessels than in uninjured vessels. In contrast, the level was significantly higher in pCMV-PCS-transfected SMCs compared with the pCMV-lacZ-transfected SMCs.
vessels than in the control and pCMV-lacZ-transfected vessels. The PCS gene transfer restored the 6-keto-PGF$_{1\alpha}$ levels to values even higher than those of the uninjured vessels ($n=6$ in each group, Figure 3). Thromboxane A$_2$ (TxA$_2$), the counterpart of derivatives from PGH$_2$, was also evaluated in the same samples by determining the levels of thromboxane B$_2$ (TxB$_2$). There were no significant differences in the TxB$_2$ levels among the groups at baseline or after arachidonate treatment.

**Effect of Arterial PCS Gene Transfer on SMC Proliferation and Neointimal Formation**

The inhibitory effect of PGI$_2$ on SMC proliferation was confirmed by BrdU incorporation rate in the vessel walls. In the control group, the BrdU incorporation index was $47.6\pm7.8\%$ ($n=5$). The PCS gene transfer significantly suppressed the BrdU incorporation to $7.5\pm3.3\%$ ($n=6$, $P<0.001$ versus control and pCMV-lacZ), whereas the lacZ gene transfer did not affect the index ($50.7\pm9.6\%$, $n=6$). To investigate whether the local delivery of the pCMV-PCS gene can accelerate the recovery of endothelium and reduce neointimal formation, we conducted a histological analysis of balloon-injured rat carotid arteries. We first evaluated reendothelialization after endothelial denudation. A prominent recovery of endothelium was observed in the pCMV-PCS-transfected arteries (Figure 4). The mean reEI at 7 days after the balloon injury was $87.1\pm6.9\%$ in the pCMV-PCS-transfected arteries ($n=6$, $P<0.001$ versus control and pCMV-lacZ), whereas the lacZ gene transfer did not affect the index ($50.7\pm9.6\%$, $n=6$). To investigate the effect of gene transfer on neointimal formation, we calculated the I/M ratio in each cross-section of artery (Figures 5 and 6). At 7 days after injury, a significant difference was observed ($n=6$ in each group, $P<0.01$ versus control and pCMV-lacZ). The data are mean$\pm$SEM.

**Protein Synthesis**

The production of 6-keto-PGF$_{1\alpha}$, the main metabolite of PGI$_2$, significantly increased in the pCMV-PCS-transfected arteries ($*P<0.05$ versus control and pCMV-lacZ). The data are means$\pm$SEM of 6 vessels per group. (B) The level of TxB$_2$. No significant difference was observed among 3 groups. Gene transfer did not affect TxA$_2$ synthesis.

**Figure 3.** Prostanoid production in the segments of rat carotid arteries. (A) The level of 6-keto-PGF$_{1\alpha}$. The production of 6-keto-PGF$_{1\alpha}$, the main metabolite of PGI$_2$, significantly increased in the pCMV-PCS-transfected arteries. $*P<0.05$ versus control and pCMV-lacZ. The data are means$\pm$SEM of 6 vessels per group. (B) The level of TxB$_2$. No significant difference was observed among 3 groups. Gene transfer did not affect TxA$_2$ synthesis.

**Figure 4.** The reEI after balloon injury. (A) No significant difference was observed between the control and pCMV-lacZ groups 7 days after balloon injury. The reEI of the pCMV-PCS group was significantly higher than in the other two groups ($n=6$ in each group, $P<0.001$ versus control and pCMV-lacZ). Similarly, 14 days after balloon injury, the reEI of the control and pCMV-lacZ groups increased up to 60%. In contrast, the endothelial regeneration was almost completed in the pCMV-PCS group and the index indicates $95.2\pm8.1\%$ ($n=6$ in each group, $P<0.01$ versus control and pCMV-lacZ). The data are mean$\pm$SEM.
In the pCMV-PCS group, 88% and 87% reductions of the I/M ratio were observed compared with the ratio of the control and pCMV-lacZ-transfected vessels. This effect was also observed at 14 days. The I/M ratios were 1.20 ± 0.14, 1.24 ± 0.15, and 0.14 ± 0.05 for control, pCMV-lacZ, and pCMV-PCS, respectively (n = 6 in each group, P < 0.001 versus control and pCMV-lacZ). In contrast, the medial layer area was 1.1 ± 0.07 mm² in the injured, untransfected arteries and was essentially unchanged after gene transfer with pCMV-lacZ (1.2 ± 0.08 mm²) and with pCMV-PCS (1.0 ± 0.03 mm², n = 6 in each group, P = NS). Intraluminal thrombus formation is often observed after mechanical stimuli such as balloon injury. No clogging with thrombus was observed in the pCMV-PCS-transfected vessels, whereas 14% of the vessels were embolized in the pCMV-lacZ-transfected group (n = 30 and 28, respectively, P < 0.05).

Discussion
The local delivery of the gene encoding PCS to balloon-injured rat carotid arteries transduced 8.5% of cells in the segment with nonviral lipofection. The overexpression of PCS increased the production of PGI₂ in transfected SMCs, and elevated the cAMP levels. We observed abundant regenerated endothelium at the injured site (87.1%) by the single PCS gene transfer and marked reduction of neointimal formation (88%) 7 days after balloon injury. This reduction remained at 14 days after injury.

Many early responses to balloon injury are initiated by platelet adhesion, aggregation, and thrombus formation followed by the adhesion and invasion of blood cells such as macrophages and lymphocytes into arterial walls. The interaction of these blood cells and vessel walls triggers the synthesis and release of various kinds of growth factors such as platelet-derived growth factor-α, transforming growth factor-β, and basic fibroblast growth factor. These growth factors stimulate SMCs to migrate from the media into the intima, where they start to proliferate and secrete extracellular matrix components. The loss of the endothelial monolayer is associated with the regulatory dysfunction of these growth factors. Moreover, it is likely that PGI₂ suppresses many of these reactions to arterial injury. Although each individual protective effect of PGI₂ may not be enough strong, a combination of such inhibitory effects may occur in multiple steps and result in marked inhibition of proliferative changes after balloon injury. This mechanism might be similar to those of other vasodilative molecules such as c-type natriuretic peptide and nitric oxide, which maintain vascular function through an autocrine/paracrine loop.

The present results showed that PCS gene transfer accelerated the regrowth of endothelium and restored PGI₂ production in injured arteries, events that may lead to the recovery of beneficial functions of endothelium such as the
inhibition of thrombus formation and intimal hyperplasia.\textsuperscript{30–32} The mechanism underlying the acceleration of reendothelialization by PG\textsubscript{I\textalpha} remains unknown. We speculate that through the interaction between PG\textsubscript{I\textalpha} and some growth factors, such as vascular endothelial growth factor and basic fibroblast growth factor, endothelial cell regrowth may be accelerated.

The vasculoprotective functions of PG\textsubscript{I\textalpha} including vasodilation, anti-platelet aggregation, anti-leukocyte adhesion to vessel wall, and SMC proliferation have been known since the 1970s,\textsuperscript{24,25} and clinical applications of PG\textsubscript{I\textalpha} to proliferative vascular diseases have been introduced.\textsuperscript{40–43} However, the clinical application of PG\textsubscript{I\textalpha} has been limited because of its intolerable systemic, mainly hemorrhagic, side effects.\textsuperscript{40–43} Regarding restenosis after angioplasty, Gershlick et al\textsuperscript{40} reported that a short-term administration of PG\textsubscript{I\textalpha} could not prevent restenosis after balloon angioplasty. To obtain enough effect, a higher dose of PG\textsubscript{I\textalpha} may be required inevitably accompanied by intolerable side effects. To increase PG\textsubscript{I\textalpha} production, Zoldhelyi and colleagues\textsuperscript{22} constructed a vector carrying the PGHS-1 gene and delivered it to balloon-injured arteries, resulting in the prevention of thrombus formation after balloon injury. The overexpression of the PGHS-1 gene also increased 6-keto-PGF\textsubscript{1\textalpha} levels in both cultured cells and in vessels. However, the alteration of TxA\textsubscript{2} catalyzed by the overexpression of the PGHS-1 gene was not evaluated in their report. An overproduction of PGH\textsubscript{2} may induce vascular contraction and platelet aggregation and also result in an insufficient reduction of neointimal formation.\textsuperscript{44–46} Pritchard et al\textsuperscript{47} reported that after balloon injury, PG\textsubscript{I\textalpha} production would increase because of the induction of PGHS-2 in the vessel wall even after a disturbance of constitutive PGHS-1 activity. We speculated that a sufficient supply of PGH\textsubscript{2} may induce an increased production of PG\textsubscript{I\textalpha} catalyzed by overexpressed PCS in damaged vessel walls. In support of our speculation, Shitashige et al\textsuperscript{48} investigated the mechanism of the different utilizations of arachidonic acid between PGHS-1 and PGHS-2 and reported that a small amount (<2.5 \textmu M/L) of arachidonic acid released by some stimuli is converted exclusively by PGHS-2. In the present study, the 6-keto-PGF\textsubscript{1\textalpha} levels were elevated in the arteries transfected with PCS gene without an increase in the TxA\textsubscript{2} level, suggesting that the overexpression of PCS may increase PG\textsubscript{I\textalpha} production without affecting TxA\textsubscript{2} synthesis after balloon injury. In addition, we did not observe hemorrhagic side effects locally or systemically in the PCS-gene transfected rats; this may be related to the imbalance of PG\textsubscript{I\textalpha} and TxA\textsubscript{2} production.\textsuperscript{49}

In the present study, we transfected the rat PCS gene into carotid arteries with a nonviral lipofection method, resulting in prolonged inhibition of neointimal formation. As a methodology of gene transfer, lipofection is inferior to virus-mediated transfection in expression efficiency (4% to 5% and 10% to 30%, respectively).\textsuperscript{12–23,50} However, virus-mediated gene transfection has several limitations. For example, adenovirus vectors retain most the parent virus genome, which is associated with undesired gene expression that results in both immune and vascular inflammatory responses.\textsuperscript{50} Other virus-mediated vectors such as adenov-associated virus and retrovirus have recently been developed to reduce these undesirable responses, but they have limitations regarding the convenience of preparation or with repeated usage.\textsuperscript{33,34,50} We confirmed that the liposomal reagent used in the present study had enough potential transgene efficiency (8.5% in transfected vessel cells) and markedly inhibited restenosis after arterial injury. In transgene studies, it is well known that the bystander effect is involved in the inhibition of vascular SMC proliferation.\textsuperscript{51} Our results show that as few as 8.5% of cells transfected with PCS gene affected the entire cells of the carotid artery vasoprotectively (ie, accelerated reendothelialization and inhibition of neointimal formation) through this bystander effect.

Several problems must be resolved before the clinical application of gene therapy using PCS gene transfer to prevent restenosis. We focused mainly on neointimal hyperplasia at the balloon-injured site. In humans, however, it is well known that elastic recoil (within one day after angioplasty) and shrinkage at the late phase (about 3 to 6 months) may be involved in restenosis.\textsuperscript{2–4,5–24} In this respect, further studies are required before adding gene therapy as an alternative therapeutic strategy for restenosis.

In conclusion, the local delivery of PCS gene markedly inhibited neointimal formation after balloon injury in rat carotid artery with accelerated reendothelialization. PCS gene transfer may be a novel gene therapeutic strategy for restenosis after angioplasty.

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References


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