EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 34, No. 4, 299-307, September 2002

Catheter-based adenovirus-mediated local intravascular gene delivery of a soluble TGF-β type II receptor using an Infiltrator in porcine coronary arteries: efficacy and complications

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Accepted 16 September 2002

Abbreviations: TGF-β, transforming growth factor β; ECM, extracellular matrix; AdTβ-ExR, adenovirus expressing an entire ectodomain of the TGF-β type II receptor; AdCALacZ, adenovirus expressing β-galactosidase; pfu, plaque formation units; LAD, left anterior descending artery; FITC, fluorescein isothiocyanate; LCX, left circumflex artery; RCA, right coronary artery; RT-PCR, reverse transcription PCR; X-gal, 5-bromo-4-chloro-3-indolyl-β–D-galactopyranoside

Abstract

Enhanced extracellular matrix (ECM) accumulation is an important finding in human restenotic arterial neointima after angioplasty. Transforming growth factor β1(TGF-β1) is known to regulate the synthesis and turnover of a variety of ECM components, and may play an important role in restenosis. Recombinant adenoviral vector expressing an ectodomain of the TGF-β type II receptor fused to the human immunoglobulin Fc portion (AdTβ-ExR) inhibits the action of TGF-β probably either by adsorbing TGF-β or by acting as a dominant negative receptor. We carried out a catheter-based local adenovirus mediated gene delivery using an Infiltrator in porcine coronary arteries to know the pattern of gene expression, efficacy and procedural complications. Twenty four coronary arteries in 13 pigs were used for intravascular gene delivery by intramural injection with either AdTβ-ExR or adenovirus expressing β-galactosidase (AdCA-LacZ). Direct immunofluorescent staining and reverse transcription polymerase chain reaction (RT-PCR) were used for detection of type II TGF-β receptor and its mRNA respectively. X-Gal histochemistry was performed to identify β-galactosidase. Both soluble TGF-β receptor and β-galactosidase were expressed locally in the media and adventita at injected arterial segments without any significant dissemination to remote area. Intravascular gene transfection performed with various titer of each adenoviral vector showed that AdTβ-ExR of $5x10^8$ pfu and AdCALacZ of 2.5×10^8 pfu were the minimum titer for the expression of each transgene. Infiltration of CD3 positive T cells was detected by immunohistochemical staining in the area of each transgene expression, and tends to decrease over time after gene delivery. Pathological study of 24 treated arteries showed complications such as disruption of external elastic lamina with hemorrhage (n = 4), minimal disruption of internal elastic lamina and endothelial layer, and medial thickening. In conclusion, catheter-based local intravascular gene delivery of adenoviral vector is feasible and effective in a selected artery, but must be undertaken with caution due to possible lethal complications. Local delivery of soluble TGF-β type II receptor in this way may provide an effective intravascular gene therapy to inhibit TGF-β signal pathway without any significant systemic side effect.

Keywords: gene therapy, local delivery, transforming growth factor β receptors, extracellular matrix, coronary restenosis, catheterization

Introduction

Although percutaneous coronary intervention is currently widely accepted as a treatment of coronary artery disease, restenosis rate is still relatively high. Pathological analysis of human coronary arterial restenotic neointima after stenting showed that extracellular matrix (ECM) accumulation plays an important role in neointima formation (Strauss *et al*., 1992; Chung *et al*., 2002). Transforming growth factor β1 (TGF-β1) is known as a multifunctional cytokine that regulates cell proliferation and differentiation, angiogenesis, and synthesis of a variety of ECM components such as

proteoglycans, hyaluronan, fibronectin, and collagen (Schönherr *et al*., 1991; Chen *et al*., 1993; Nabel *et al*., 1993; Schönherr *et al*., 1993; Dijike *et al*., 1994; Yamamoto *et al*., 1996; Schulick *et al*., 1998; McCaffrey *et al*., 2000). And its expression was significantly higher in restenotic lesions compared with primary lesions (Nikol *et al*., 1992). Blocking TGF-β1 activity with neutralizing antibody fol-lowing vascular injury decreases ECM accumulation and suppressed intimal hyperplasia in the rat model of arterial injury (Wolf *et al*., 1994). Most cells have three types of TGF-β receptors (type I, type II, and type III) at the cell surface, and both type I and type II receptor are necessary for all tested biological activity of TGF-β, whereas type III is not known to mediate any of the known biological activity of TGF-β (Chen *et al*., 1993; Dijike *et al*., 1994). Type II receptor is a member of the transmembrane receptor serine-threonine kinase family, and TGF-β signals through a heteromeric complex between the type I and type II receptors (Chen *et al*., 1993; Bassing *et al*., 1994; Dijike *et al*., 1994). Binding of TGF-β to the type I receptor requires the presence of the type II receptor, whereas type II receptor binds TGF-β independently (Bassing *et al*., 1994; Dijike *et al*., 1994). Type I receptor requires the presence of a functional type II TGF-β receptor to signal, and trans-phosphorylation of the type I receptor by the type II receptor seems to be essential for signaling (Bassing *et al*., 1994; Yamamoto *et al*., 1996).

As a consequence of developments in the field of molecular biology, strategies targeting TGF-β has been recently developed. For example, ribozyme oligonucleotides cleave the mRNA of TGF-β, leading to decreased expression (Yamamoto *et al*., 2000). An adenoviral vector expressing the ectodomain of the type II TGF-β receptor (AdTβ-ExR) inhibits the action of TGF-β both *in vitro* and *in vivo* (Smith *et al*., 1999; Sakamoto *et al*., 2000). This soluble receptor is also known to be able to reach remote areas by means of systemic circulation. Recent animal trials using either ribozyme oligonucleotides or soluble type II TGF-β receptor to inhibit TGF-β in vascular injury model showed beneficial effects by inhibiting either neointima or negative vascular remodeling (Smith *et al*., 1999; Yamamoto *et al*., 2000; Kingston *et al*., 2001). These studies used different gene transfer methods including systemic intravenous injection (Smith *et al*., 1999), retention of agents in surgically isolated vascular segment (Yamamoto *et al*., 2000), or Infilt-rator (Kingston *et al*., 2001). Local delivery of agent without any significant systemic untoward effect would be preferred to systemic injection for gene therapy in the local vascular lesion. Gene transfection using channeledballoon angioplasty catheter which allows low pressure local agent delivery through perforated channels has low efficiency in gene delivery (Feldman *et al*., 1996). Thus it is conceivable that gene transfection by transient retention of adenovirus in the isolated vascular segment probably has very low gene transfection efficiency. The Infiltrator is a balloon catheter with 21 injector ports in three lines designed for direct intramural injection of the desired agent. Previous studies using an Infiltrator, in general, agree that it is an effective tool for intravascular drug or gene delivery, but knowledge about efficacy of gene expression at different titer of virus vector and about procedural complication is limited.

Since local blockade of TGF-β seems to have therapeutic potential in the vascular pathology, we carried out catheter-based adenovirus mediated local intravascular delivery of a soluble TGF-β type II receptor using an Infiltrator in a porcine coronary arterial model to know about the expression pattern of this transgene, inflammatory reaction, feasibility and complications of catheter-based gene therapy. And we also tried to identify the minimum titer of virus vector with which the soluble receptor can be expressed in the selected arterial segment without any significant dissemination into remote areas.

Materials and Methods

Recombinant adenovirus vectors

Replication-defective E1- and E3- recombinant adenovirus expressing either an entire ectodomain of the TGF-β type II receptor fused to the human immunoglobulin Fc portion (AdTβ-ExR) or β-galactosidase (AdCALacZ) under a CA promoter, composed of cytomegalovirus enhancer and chicken β-actin promoter, was constructed as described previously (Ueno *et al*., 1995; Qi *et al*., 1999). TGF-β type II soluble receptor is known to be secreted from AdTβ-ExR-infected cells, binds to TGF-β and inhibits TGF-β signaling (Sakamoto *et al*., 2000). Both AdTβ-ExR and AdCALacZ were used as adenoviral vectors for gene delivery in porcine coronary arteries. The titer of virus was quantified by a plaque formation assay using 293 cells and expressed as plaque formation units (pfu).

Local gene delivery at porcine coronary artery

Thirteen domestic female pigs (2-3 month old, weighing 25-30 kg) underwent coronary artery intravascular gene delivery using an Infiltrator (Interventional Technologies, San Diego, CA). All animal care and handling were performed in accordance with the guideline specified by the National Institute of Health Guide for the Care and Use of Laboratory animals and were approved by the Animal Care and Use Committee of Yonsei University. Animals took 100 mg of aspirin and 75 mg of clopidogrel 24 h before procedure and continued to take these medication until sacrifice. Before anesthesia, pigs subjected intramuscular injection of atropine (0.04 mg/kg) and rompun (2 mg/kg). After endotracheal intubation, anesthesia was induced by inhalation of 2.5% enflurane. Heparin (3,000 IU) was injected intravenously

Table 1. Results of gene delivery using an Infiltrator and subsequent stent deployment in porcine coronary arteries. No, number; AdTβ-ExR, adenovirus vector expressing soluble TGF-β type II receptor; AdCALacZ, adenovirus vector expressing β-galactosidase; PBS, phosphate buffered saline; ϕ, diameter; atm, atmosphere; Di, dissection; FD, failure of deballooning; Sp, spasm; VT, ventricular tachycardia

No	Sites	Vector	Titer (Pfu)	Lumen Φ (Mm)	Complication	Outcome
1	m-LAD	AdTβ-ExR	$1x10^8$	3.0		alive
	m-RCA	AdCALacZ	$2.5x10^7$	3.5		
2	m-LAD	AdTβ-ExR	$5x10^8$	3.0		alive
	p-LCx	AdCALacZ	$2.5x10^8$	3.5	Di	
3	p-LAD	AdTβ-ExR	$5x10^8$	3.5		alive
	d-LAD	AdTβ-ExR	$1x10^9$	3.5		
4	p-RCA	AdTβ-ExR	$1x10^9$	3.5		
	p-LAD	AdCALacZ	$1x10^9$	3.5	Di/Sp/VT	death
5	p-LCx	AdTβ-ExR	$1x10^9$	3.5		alive
	p-RCA	AdCALacZ	$1x10^9$	3.5		
6	m-LAD	AdTβ-ExR	$1x10^9$	3.0		alive
	d-RCA	AdCALacZ	$1x10^9$	3.0		
7	p-LAD	AdTβ-ExR	$1x10^9$	3.0	Di/Sp/VT	death
8	m-LAD	AdTβ-ExR	$1x10^9$	3.0		alive
	m=RCA	PBS		3.0		
9	m-LAD	AdTβ-ExR	$1x10^9$	2.5		alive
	d-RCA	PBS		2.5	Sp/VT	
10	m-RCA	AdTβ-ExR	$1x10^9$	3.0	Sp	alive
	d-LCx	PBS		3.0		
11	p-RCA	AdTβ-ExR	$1x10^9$	3.0	Sp	alive
	p-LAD	PBS		3.0		
12	p-LCX	AdTβ-ExR	$1x10^9$	3.0	FD/Sp/VT	death
13	m-LAD	AdTβ-ExR	$1x10^9$	3.0		alive
	d-RCA	PBS		3.5		

before coronary arterial intervention. An 8 F Judkins right coronary artery guide catheter was inserted through the left carotid artery. Coronary arterial segments in the left anterior descending (LAD), left circumflex (LCX), or right coronary artery (RCA) feasible for intravascular gene delivery using an Infiltrator (3.0-3.5 mm) were selected. An infiltrator was used for injection of 400 μl of each adenoviral vector following inflation of the Infiltrator to 2 atmosphere. Coronary angiogram was performed at both before and immediately after gene delivery. Intracoronary injection of isosorbide dinitrate (200 μg) was performed before angiogram to relieve any possible vasospasm. To identify optimal titer of adenoviral vector for effective gene transfer, three different titers of AdTβ-ExR (1×10^8, 5×10^8, or 1×10^9 pfu in 400 μl PBS) and two different titers of AdCALacZ (2.5×10^7 or 2.5×10^8 pfu in 400 μl PBS) were randomly injected into each coronary arterial segment in three pigs (No. 1-3 in Table 1). The rest of pigs undertook intravascular injection of adenoviral vector or PBS as shown in Table 1. After gene delivery, catheters were removed, and left carotid artery was ligated.

To identify the efficiency of gene transfer, three pigs (No. 1-3 in Table 1) were sacrificed at 1 week after gene delivery. Three pigs (No. 4, 7, 12 in Table 1) died during the procedure due to complications, and seven pigs were sacrificed at 4 weeks after gene delivery. Coronary arterial segments from each adenovirus injected site were retrieved. Thoracic aorta as well as remote coronary arterial segments were used as negative control tissues. Coronary arteries dissected at 1 week after gene delivery were snap-frozen in liquid nitrogen, and were divided into two pieces: one embedded in OCT for cryostat sectioning and the other kept in 70°C until RNA assay. Arterial specimens dissected from the rest of pigs were pressure-fixed *in situ* with 4% formaldehyde, and embedded in paraffin.

Histochemical analysis for β-galactosidase

Pigs were sacrificed 1 week after gene delivery with a lethal dose of sodium pentobarbital. Coronary arterial segments were excised, fixed in PBS containing 2% formaldehyde and 0.2% glutaraldehyde for 2 h at 4°C, embedded in OCT compound, and subjected to cryostat section at 20 μm thickness. Expression of β-galactosidase was evaluated by incubation at 37°C with the substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; Sigma, St. Louis,

MO). Tissue sections were subsequently counterstained with hematoxylin-eosin.

Reverse transcription PCR (RT-PCR)

Total cellular RNA from each arterial segments was isolated using RNeasy mini kit (Qiagen, Germany). For reverse transcription and PCR reactions, a Perkin-Elmer/Cetus DNA thermal cycler (Foster city, CA) was used. Two microgram of total RNA was reverse transcribed in a 25 μl reaction containing 5 μl reverse transcriptase buffer, 25 mM dNTPs, random hexamers (0.5 μg/μl), 20 units RNase inhibitor and 200 units MMLV reverse transcriptase at 37°C for 1 h followed by 5 min at 70°C. PCR amplification was performed in a 50 μl volume using 2 μl of the reverse transcription reaction plus 1 unit of Taq polymerase, 10 x buffer, 0.2 mM dNTPs, 2 mM $MgCl_2$ and 0.2 μM of each primer under following conditions: 94°C for 3 min; followed by 35 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min; followed by 7 min at 72°C. The primer sequences and the expected product sizes were as follows: TGF-β type II receptor: sense primer 5'-ACATCGTCCTGTGG-ACGCGTA-3', antisense primer 5'-CTAGCAACAAGTC-AGGATTGC-3' with an expected size of 450 bp; and 28 S RNA: sense primer 5'-TTAAGGTAGCCAAATGCCTC-G-3', antisense primer 5'-CCTTGGCTGTGGTTTCGCT-3' with an expected size of 102 bp. PCR products were separated on a 1.2% agarose gel, stained with ethidium bromide, and analyzed using an image analyzer (Bioprofil, Viber Lourmat, France).

Immunostaining

Identification of soluble TGF-β type II receptor was assessed by using direct immunofluorescent staining to detect human IgG fused to this soluble receptor with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human IgG (Dako, Carpinteria, CA). Briefly, frozen sections were dried, placed in PBS for 10 min, incubated with FITC-conjugated rabbit anti-human IgG at room temperature for 1 h, rinsed with PBS for 10 min, and mounted with glass coverslip. The immunostained slides with FITC conjugated anti-human IgG complex were assessed by using immunofluorescent microscope, and photographed immediately. T cells were identified in specimens taken at both 1 week and 4 weeks after gene delivery by immunohistochemical staining with rabbit polyclonal anti-human CD3 antibodies (Dako).

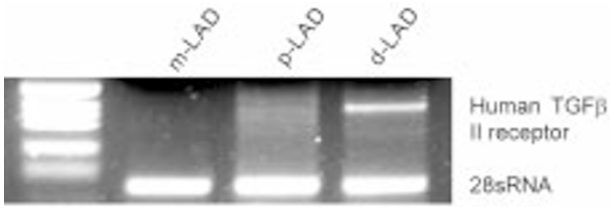

Figure 1. RT-PCR analysis for detection of a transferred soluble TGF β type II receptor mRNA in each porcine coronary artery. RT-PCR analysis showed the amplified gene product from the distal LAD injected with $1x10^9$ pfu AdTβ-ExR. Proximal LAD injected with $5x10^8$ pfu AdTβ-ExR showed a faint band of the soluble receptor gene product, whereas mid LAD in between two injection sites showed no transgene product. Note the bands of 28S RNA product from each arterial segment which verify a quantitative analysis of human TGF-β type II receptor gene product.

Results

Identification of human TGF-β type II receptor gene

Three pigs were sacrificed 1 week after gene delivery to analyze the efficiency of gene transfection. As shown in Figure 1, RT-PCR products from porcine coronary artery (d-LAD) injected with AdTβ-ExR of 1×10^9 pfu showed a definite band of human TGF-β type II receptor gene product, while artery (p-LAD) injected with that of 5×10^8 pfu showed a faint band of the gene product. Neither arterial segment adjacent to AdTβ-ExR (1×10^9 pfu) injected site (m-LAD), as shown in Figure 1, nor artery injected with AdTβ-ExR of 1×10^8 pfu (data not shown) revealed any detectable band. These results suggest that

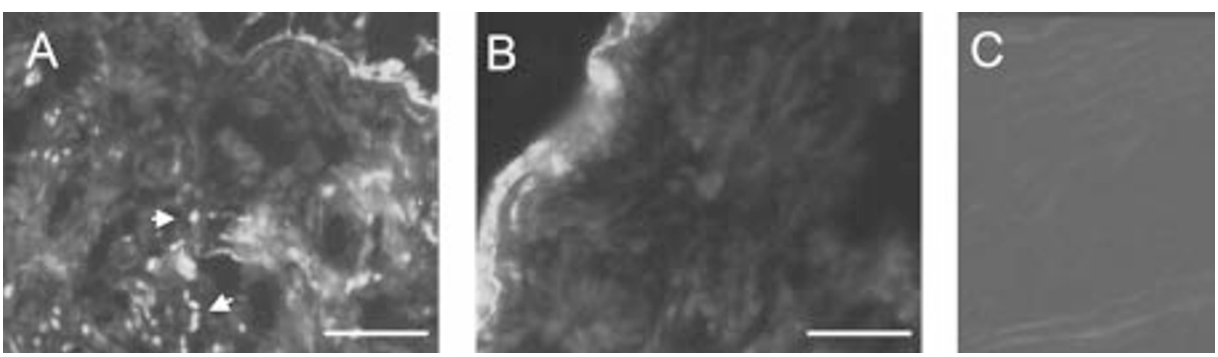

Figure 2. Immunohistochemical staining of porcine coronary arteries for detection of soluble TGF β type II receptor with FITC-conjugated rabbit anti-human IgG. A) There are multiple dispersed tiny immunofluorescent positive particles (marked as arrows) of soluble TGF β type II receptor located in media and adventitia of the distal LAD injected with 5×10^8 pfu AdTβ-ExR. (B) and (C), Right coronary artery in the same pig, remote from distal LAD, shows no discernable soluble receptor: with (B) and without (C) FITC conjugated antibody, bar = 50 μm.

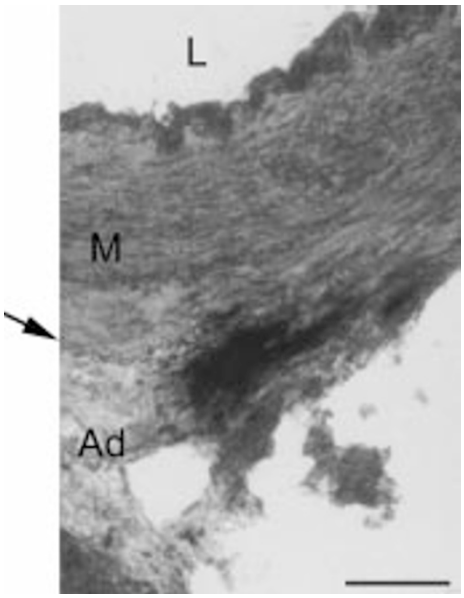

Figure 3. Porcine coronary artery injected with $2.5x10^8$ pfu AdCALacZ shows β-galactosidase activity identified by histochemical staining with the substrate X-gal. Note the blue stained area showing the expression of β-galactosidase in the outer media and adventitia. Counter staining with hematoxylin-eosin. Arrow head shows external elastic lamina. Ad, adventitia; L, lumen; M, media; bar=50 μm.

successful local gene delivery of human TGF-β type II receptor was carried out using an Infiltrator without any significant dissemination of adenovirus vector. RT-PCR products of 28S RNA from each artery showed a band of equal density, which verify a quantitative analysis of human TGF-β type II receptor gene product.

Identification of soluble receptor

Direct immunofluorescent staining with use of FITC-conjugated rabbit anti-human IgG identified the soluble TGF-β type II receptor, and, as shown in Figure 2, coronary artery injected with 5×10^8 pfu AdTβ-ExR showed multiple dispersed immunofluorescent positive soluble receptor-antibody complex particles in around media and adventitia. The soluble TGF-β receptor was more widely dispersed comparing with the distribution of β-galactosidase. On the other hand, remote arteries including remote coronary artery and thoracic aorta showed no discernable immunofluorescent positive particles. Thus, it is conceivable that the soluble receptor delivered by certain range of adenoviral vector could be expressed locally at the site of gene delivery without any significant spread to remote sites.

Identification of β-galactosidase

Gene transfection was also studied by immunohistochemical assay of β-galactosidase in the arteries injected with two different titers of AdCALacZ (2.5×10^7 pfu and 2.5×10^8 pfu). As shown in Figure 3, the expression of β-galactosidase was noted locally in around outer media and adventitia at the arterial segment injected with AdCALacZ of 2.5×10^8 pfu, and there was no significant spread of β-galactosidase at remote arterial segments. Coronary arterial segments injected with 2.5×10^7 pfu AdCALacZ had no expression of β-galactosidase (data not shown).

Inflammation

$CD3^+$ T cells were infiltrated around adventitia and, with less extent, media in arteries 1 week after injection with either AdCALacZ or AdTβ-ExR (Figure 4). More T cells were infiltrated in segments injected with AdCALacZ comparing to that with AdTβ-ExR. The numbers of infiltrated T cells tend to decrease over time after gene delivery, and there were only a few T cells present in arteries 4 weeks after injection of adenovirus vector (data not shown). The remote coronary artery has no $CD3^+$ T cells as shown in Figure 4.

Procedural complications

As shown in Table 1, ten pigs survived after catheter-

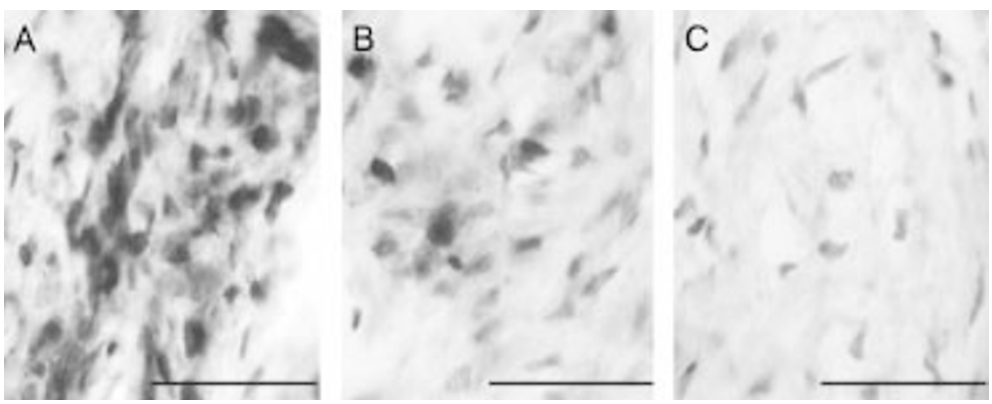

Figure 4. Infiltrated $CD3^+$ T cells in the porcine coronary arteries injected with each adenovirus vector. Arteries 1 week after injection with either AdCALacZ ($2.5x10^8$ pfu) (A) or AdTβExR ($5x10^8$ pfu) (B) show $CD3^+$ T cells (brown color) infiltrated in adventitia, whereas remote control artery (C) has no $CD3^+$ cells. Counterstaining with hematoxylin; bar = 50 μm.

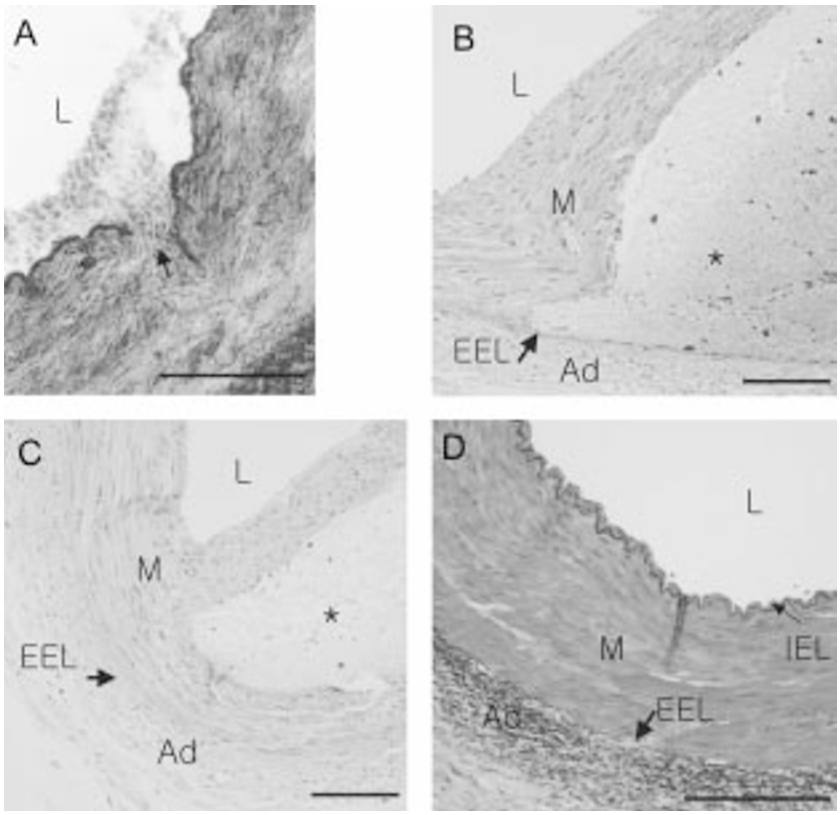

Figure 5. Procedural complications in porcine coronary arteries treated with Infiltrator**.** (A) Rupture (marked as an arrow) of intima and underlying internal elastic lamina in a porcine coronary artery 1 week after injection of adenovirus. Note the nest of cells in the loose ECM protruded into the lumen through this ruptured layers. (B) and (C) Cases of rupture occurred in external elastic lamina (B) and media (C) in the arteries treated with Infiltrator. Note the hemorrhagic fluid (*) accumulation in the ruptured space. (D) Normal porcine coronary artery stained with Movat pentachrome staining. Ad, adventitia; IEL, internal elastic lamina; EEL, external elastic lamina; L, lumen; M, media. Hematoxylin-eosin staining (A, B, C); bar = 100 μm.

based gene delivery. Total three pigs died during the procedure. Two pigs died due to ventricular tachycardia induced by intimal dissection and subsequent total occlusion of the artery. Pathological study of these arteries revealed disruption of either external elastic lamina or, less frequently, of tunica media with hemorrhage in ruptured space (Figure 5B and 5C). Minimal dissection at the external elastic lamina without any significant compromise of coronary circulation was noted in one artery. In addition, interruption of the endothelial layer and internal elastic lamina, miniscule channel penetrating into media with protrusion of nest of cells in a loose ECM, medial thickening caused by edema, and interrupted medial layer were also noted (Figure 5A). One pig died due to the failure of deballooning of the Infiltrator and subsequent intravascular occlusive thrombosis. Transient coronary arterial spasm responding to intracoronary nitrate injection occurred in three arterial segments. Ventricular tachycardia associated with arterial spasm were often fatal in our study: only one of four pigs with ventricular tachycardia survived after direct current cardioversion.

Discussion

We studied the efficacy and complications of adenovirus mediated local intravascular delivery of a soluble TGF-β type II receptor using an Infiltrator in a porcine coronary arterial model known to be very similar to that of human (Muller *et al*., 1992). Expression of β-galactosidase delivered by an Infiltrator was mostly localized around outer media and adventitia, which seems to be related with the height (279 μm) of injector ports. And similar observations were reported by other studies (Morishige *et al*., 2000; Kingston *et al*., 2001). Our study suggested that no significant dissemination of adenovirus occurred, since transgene, ectodomain of human TGF β type II receptor mRNA, was localized in the injection segment. And this finding is consistent with the previous study (Feldman *et al*., 1995). The soluble TGF-β receptor delivered by injection of 5×10^8 pfu AdTβ-ExR was more widely dispersed in around media and adventitia comparing with that of β-galactosidase, but neither TGF-β receptor nor β-galactosidase was found at remote arterial segments. The same soluble

receptor is capable to reach remote areas by means of systemic circulation in other study. Intramuscular injection of same amount of AdTβ-ExR (5×10^8 pfu) in a mouse showed that an increase of concentration of this soluble receptor was noted not only in the serum, but also in the remote organ (Sakamoto *et al*., 2000). This discrepancy is probably caused by the difference of dilution of soluble TGF-β receptor between two different animal models. Our animal model (pig, ≅30 kg) which is much larger than that of the other study (mouse, ≅30 g) should enable the soluble receptor to dilute significantly in the systemic circulation.

A replication-defective adenoviral vector has several advantages over other vectors for *in vivo* gene transfer to intravascular model. The adenovirus vectors, unlike the retroviruses, are effective in delivering gene in non-dividing vascular cells (Wivel *et al*., 1998). Vascular cellular replication rate is known to be very low (mostly 0-1%) in human arterial tissue, which is true in human restenotic arterial tissue as well as in primary atherosclerotic plaque (Gordon *et al*., 1990; OBrien *et al*., 1993; Chung *et al*., 2002). Cell replication rate is also known to be low (≤ 5%) in injured rabbit artery (Strauss *et al*., 1994) and in our porcine coronary stent neointima specimens (unpublished data). In addition, adenoviral vectors are stable in the blood stream, and adenoviral DNA is not integrated into the host cell chromosome.

An adenoviral vector expressing the ectodomain of the type II TGF-β receptor (AdTβ-ExR) inhibits the action of TGF-β by adsorbing TGF-β, but it may act as a dominant negative receptor (Sakamoto *et al*., 2000). As a possible mechanism of dominant negative mutation, Chen *et al*. suggested that truncated and wild type receptor form heterodimers and abrogate further sig-naling via either accelerated degradation of the hetero-dimers or impaired transport of the wild type receptor to the cell surface that accounts for reduced level of endogenous type II receptor. In addition, truncated type II receptor may form a heterodimer with type I receptor preventing an interaction of the endogenous type II receptor and type I receptor. Therefore, it is noteworthy that due to the intrinsic competitive nature of dominant negative mutants the expression level of soluble receptor needs to greatly exceed that of the endogenous receptor (Chen *et al*., 1993).

Host immune response and dose-dependent inflammatory and cytopathic effects inhibit adenoviral gene expression, and impose limitations in gene delivery (Grub *et al*., 1994; Feldman *et al*., 1995; Wivel *et al*. 1998). Therefore identification of viral titer that is effective in gene expression with minimal inflammatory reaction seems to be important. Gene transfer assay with different titers of each adenoviral vector showed that the minimum adenoviral titers for effective gene expressions were 5×10^8 pfu for AdTβ-ExR and 2.5×10^8 pfu for AdCALacZ in our study. And arterial segments 1 week after adenoviral vector mediated gene delivery showed $CD3^+$ T cells infiltrated in the area of adventitia and media, and the numbers of infiltrated T cells tend to decrease over time. This is consistent with other study that intramuscular injection of E1 deleted adenoviral vector expressing β-galactosidase in a mice induced a significant inflammatory response characterized by mononuclear cell infiltration and degeneration of muscle fiber (Yang *et al*., 1996). This study also showed that inflammation, peaked at 17 days after gene transfer, slowly diminished to baseline over several months, and that the expression of transgene diminished to undetectable level within 30 days. Cellular immunity dependent on $CD4^+$ and $CD8^+$ T cells limit the duration of vector derived transgene expression, and humoral immunity also plays a role in diminishing gene transfer following a second administration of vector (Yang *et al*., 1994; Yang *et al*., 1995; Yang *et al*., 1996). Activation of $CD8^+$ T cells is known to be induced by viral capsid proteins, newly expressed viral proteins, and the transgene product (Yang *et al*., 1996). In our study, infiltrated CD3 positive T cells were more in segments injected with AdCALacZ comparing to that with AdTβ-ExR. This finding is consistent with other study (Kingston *et al*., 2001), and should be considered in the context with the concept that β-galactosidase has the potential for eliciting immune responses (Danko *et al*., 1994; Yang *et al*., 1996).

The Infiltrator was developed as a tool for local intravascular delivery of drug, and has been used in animals (Barath *et al*., 1997; Varenne *et al*., 1998; Morishige *et al*., 2000; Kingston *et al*., 2001) and rarely in human (Pavlides *et al*., 1997). Our study showed that catheter-based local *in vivo* adenovirus mediated gene transfer using an Infiltrator is, in general, effective and feasible in an arterial segment characterized by diameter of at least 2.5 mm, light tortuosity, minimal angulation (< 45°), and free of side branch. However procedure-related complications such as medial dissection with hemorrhage and subsequent vasospasm occurred in 4 arteries among 24 treated arteries in our study. We occasionally observed that some injector ports of Infiltrator were obliterated after use, which may be a possible cause for the hydraulic dissection due to excess of fluid accumulation at the limited vascular space. Therefore further studies aimed for the improvement of feasibility and safety are necessary before the appli-cation in human.

Acknowledgments

We appreciated for excellent advice given by Dr. Stephen M Schwartz. This work was supported by Korea Research Foundation Grant (KRF-2000-003-F00030).

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