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Stroke 2002;33:1376-1382

DOI: 10.1161/01.STR.0000014327.03964.C0

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ISSN: 1524-4628

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Endovascular Microcoil Gene Delivery Using Immobilized Anti-adenovirus Antibody for Vector Tethering

John M. Abrahams, MD; Cunxian Song, PhD; Suzanne DeFelice, BS; M. Sean Grady, MD;
Scott L. Diamond, PhD; Robert J. Levy, MD

Background and Purpose—Endovascular microcoils are widely used in interventional procedures to treat cerebral aneurysms. In the present study we report for the first time successful use of an endovascular microcoil as a gene delivery system.

Methods—Anti-adenoviral monoclonal antibodies were covalently attached to the collagen-coated surface of either platinum or polyglycolic acid microcoils. These antibodies were used to tether replication-deficient adenovirus (Ad-GFP [encoding green fluorescent protein] or Ad-LacZ [encoding β -galactosidase]). Cell culture studies with rat arterial smooth muscle cells (A10) assessed transduction on or near the coil. Platinum coils coated with Ad-GFP were implanted into the ligated common carotid artery (CCA) of adult rats in a model of arterial stasis and pressurization. After 7 days, CCA segments were harvested, and coils were removed for histopathology and GFP expression studies, while organs were evaluated by polymerase chain reaction to assess viral biodistribution.

Results—In cell culture studies, GFP-positive smooth muscle cells were detected only on the platinum coil surface, while LacZ-positive cells were detected only on the polyglycolic acid coil surface, thus demonstrating localized gene delivery. After 7-day implantation, GFP (according to fluorescence microscopy and confirmed with immunohistochemistry) was detected on the harvested platinum coil and in the organizing thrombus within the CCA but not in the arterial wall. Morphometric analyses revealed that 13.3±2.0% of cells within the organized thrombus were transduced with Ad-GFP via the gene delivery system. However, arterial smooth muscle cells were negative for GFP according to fluorescence microscopy and immunohistochemistry. Ad-GFP was not detectable by polymerase chain reaction in lung, liver, or kidney.

Conclusions—It is concluded that catheter deployment of platinum or biodegradable gene delivery endovascular microcoils represents an interventional device-based gene therapy system that can serve as a suitable platform for either single or multiple gene therapy vectors. (*Stroke*. 2002;33:1376-1382.)

Key Words: aneurysm ■ embolization, therapeutic ■ gene therapy

Endovascular microcoil occlusion of cerebral aneurysms most often uses platinum Guglielmi detachable coils (GDCs). These interventional devices cause intra-aneurysmal thrombosis, clot organization, and eventual aneurysm occlusion. However, clinical series regarding occlusion rates after GDC embolization have shown that they are less effective in wide-necked and larger aneurysms.¹⁻⁵ The largest necropsy series of 17 human aneurysms treated with coil embolization highlighted some of the limitations over long-term follow-up.⁶ Bavinski et al⁶ showed that endothelialization of the aneurysm neck occurs in the minority of cases, and as aneurysm size increases, open spaces between the coils are apparent, with incomplete membranes over the aneurysm ostium. They found that 50% of aneurysms thought to be 100% occluded by angiography were not completely oc-

cluded by histopathological evaluation. These limitations raise questions concerning the efficacy of GDC embolization.

GDCs are made from platinum, which is relatively biologically inert. Recent advances in biomaterials and biotechnology have generated interest in modifying materials from which endovascular coils are constructed to enhance their effects in achieving aneurysm closure. Research in this field has focused on modifying the surface of platinum microcoils with extracellular matrix proteins, radiation, and engineered cells to enhance overall device effectiveness.⁷⁻¹⁴ Recent research by our group has explored the concept of incorporating a controlled-release drug delivery system onto the surface of endovascular microcoils for sustained local administration of selected therapeutic agents. These studies successfully demonstrated that coil-based local delivery of recombi-

Received June 18, 2001; final revision received January 11, 2002; accepted January 22, 2002.

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Stroke is available at <http://www.strokeaha.org>

DOI: 10.1161/01.STR.0000014327.03964.C0

nant human vascular endothelial growth factor (rhVEGF) enhanced endoluminal thrombus and thus could hypothetically provide an improved outcome for coil-occluded aneurysms.¹⁵ However, the delivery of recombinant proteins has a number of limitations, including loading capacity and protein denaturation concerns. In separate studies,¹⁶ we have demonstrated that antiviral antibody can be chemically coupled onto the surface of a collagen-coated, steel coronary stent. An adenovirus vector was successfully delivered into coronary arteries with highly localized gene expression from the collagen-coated stent through a mechanism involving antiviral antibody tethering.¹⁶ Thus, we hypothesize that an endovascular microcoil could also be used as a gene delivery system, ultimately delivering therapeutic genes to facilitate coil-occlusion procedures and even to treat underlying vascular disease.

In this study we sought to formulate gene delivery systems onto the surface of a GDC using immobilized anti-adenoviral antibodies to tether the vector, thereby delivering transgenes to modify cells in the arterial wall and invading the coil-localized thrombus. The purpose of this investigation was to formulate a collagen-based coating for endovascular coils that could be used for covalently linking anti-adenoviral antibodies. Antibody-activated coils were then investigated for their potential to tether adenoviral gene vectors and deliver transgenes to vascular cells, both in cell culture and in a rat model of common carotid artery (CCA) ligation, with coil deployment. These studies investigated both platinum coils and a novel biodegradable polyglycolide coil as adenoviral vector delivery systems.

Materials and Methods

Materials

GDCs were a gift from Target Therapeutics (Freemont, Calif). Bovine dermal type I collagen (Vitrogen 100, 3.0 mg/mL in 0.012N HCl solution) was obtained from Vitrogen Cohesion Technologies. Anti-knob mouse monoclonal F(ab)² was provided by Selective Genetics. A replication-defective adenovirus (type V, E1, E3 deleted) encoding the green fluorescent protein (Ad-GFP) under the influence of the human cytomegalovirus promoter was purchased as a stock solution (5×10^{12} particles per milliliter or 1×10^{11} plaque-forming units per milliliter) from the Institute for Human Gene Therapy of the University of Pennsylvania (Philadelphia). A type 5 adenovirus (H5.010) β -galactosidase replication-defective adenovirus was also provided by the Institute for Human Gene Therapy of the University of Pennsylvania. *N*-Succinimidyl-3-(2-pyridyldithiol)-propionate (SPDP) was obtained from Pierce Chemical. A rat arterial smooth muscle cell line (A10) was obtained from American Tissue Type Collection. A nonspecific F(ab)² fragment of mouse anti-rat IgG was obtained from Pierce Chemical. Vectashield Mounting Medium for fluorescence with 4-6-diamidino-2-phenylindole (DAPI) was purchased from Vector Laboratories, Inc. Dimethyl sulfoxide was obtained from Fisher Chemicals. Dulbecco's PBS was purchased from Gibco BRL. Monoclonal anti-vimentin (mouse IgM isotype) recognizing an epitope of the cytoskeletal protein vimentin was obtained from Sigma. Rabbit anti-human von Willebrand factor/horseradish peroxidase antibody was purchased from DAKO A/S. The monoclonal mouse anti-leukocyte common antigen, CD45, clones PD7/26 and 2B11, and the monoclonal anti-human smooth muscle actin (mouse IgG2a isotype) antibody were obtained from DAKO Co, as was nonimmune (nonspecific) control IgG. The mouse monoclonal antibody to GFP 3E6 was obtained from Molecular Probes, Inc. All were visualized with the use of standard secondary antibody kits and DAB substrate from

Vector Labs, Inc. Dual immunohistochemistry staining used tetramethylbenzidine (Kirkegaard & Perry) as a second peroxidase substrate, counterstaining with nuclear fast red (Vector Labs).

Collagen Coating on Platinum and Biodegradable Polyglycolide Coils

Under sterile conditions, GDCs and biodegradable polyglycolide coils were sectioned into 4-mm segments. The Vitrogen 100 collagen solution was neutralized to pH 7.4 according to the manufacturer's instruction. Coil segments were immersed in the neutralized type I collagen solution (2.4 mg/mL collagen) and incubated at 37°C for 1 hour to allow for gelation and then air-dried in a sterile laminar flow hood for 1 hour.

Platinum Coil Modification With Adenovirus

The detailed procedure for linking anti-adenovirus antibody onto collagen coatings was described previously.¹⁶ Briefly, each collagen-coated coil was reacted with a bifunctional cross-linker, SPDP, at a concentration of 20 mmol/L in dimethyl sulfoxide/PBS (1:3 vol/vol) solution. After they were rinsed with copious PBS, the SPDP-activated coils were put into a solution of 5 μ g of the anti-adenovirus knob (Fab)² in 100 μ L PBS buffer and were incubated at room temperature overnight. Each coil was rinsed with PBS to remove unbound antibody. A replication-defective adenovirus (type V, E1, E3 deleted) for expression of the green fluorescent protein (GFP) driven by a cytomegalovirus promoter (Ad-GFP) was used as a model vector. The antibody-bound collagen-coated coils were added to Ad-GFP solution in PBS (1×10^7 plaque-forming units per coil segment) and incubated at 37°C for 1 hour. Coils were not removed from the adenovirus solution and were stored at 4°C until use. Before use, coils were washed extensively with $1 \times$ PBS.

In Vitro Transfection With Adenovirus-Linked Platinum Coils

Coils were incubated for 1 hour (37°C) in 6-well cell culture plates in 2 mL of complete media containing Medium 199 (M199, Gibco BRL), 10% fetal bovine serum (HyClone Laboratories), and 1% penicillin-streptomycin (Gibco BRL). The medium was removed, and 1×10^5 rat A10 smooth muscle cells in 2 mL of complete media were added to each well and incubated at 37°C for 3 days until confluent. Each well containing a coil was rinsed 3 times with $1 \times$ PBS followed by the addition of 2 mL of 4% paraformaldehyde (pH 7.4) (Aldrich Chemical) for fixation. After 3 minutes of fixation, wells were washed twice with PBS and then equilibrated in 2 mmol/L MgCl₂ before GFP imaging.

Biodegradable Coil Modification With Adenovirus

The collagen-coated polyglycolide coil segments, prepared as previously described,¹⁷ were reacted with the anti-knob F(ab)² in the same way as described above. Since the polyglycolide material is intensely autofluorescent, a replication-defective adenovirus (type V, E1, E3 deleted) encoding β -galactosidase (Ad-LacZ) under the control of the cytomegalovirus promoter (Institute for Human Gene Therapy, University of Pennsylvania), which is suited for chromogenic staining, was used as a marker gene instead of GFP. The anti-knob activated coil was incubated with the Ad-LacZ gene in $1 \times$ PBS buffer solution (1×10^7 plaque-forming units virus per 1 mg polyglycolide coil) at 37°C for 1 hour to permit antibody binding of the vector. Coils were washed extensively with $1 \times$ PBS before use.

In Vitro Cell Transduction of Ad-LacZ Tethered-Polyglycolide Coils

In vitro transfection experiments with Ad-LacZ-tethered biodegradable polyglycolide coils and with coils lacking Ad-LacZ exposure were performed according to the following procedure. All coils were incubated for 1 hour at 37°C in 6-well cell culture plates in 2 mL of complete media. The medium was removed, and 1×10^5 rat A10 smooth muscle cells in 2 mL of complete media were added to each well and incubated at 37°C for 3 days until confluent. Each well was

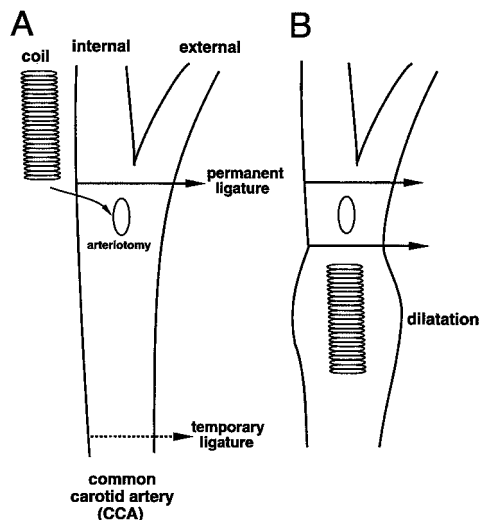


Figure 1. A, Exposure of the CCA shows temporary proximal and permanent distal ligatures. B, The coil has been inserted into the CCA, a permanent ligature has been placed proximal to the arteriotomy, and the temporary ligature has been released.

rinsed 3 times with PBS followed by the addition of 2 mL of 4% paraformaldehyde (pH 7.4; Aldrich Chemical) for 3 minutes. Wells were rinsed twice with 1× PBS and then equilibrated in 2 mmol/L MgCl₂ before X-gal staining. Two milliliters of X-gal staining solution was added to each well and incubated at 37°C overnight.

In Vivo Transfection With Ad-GFP–Tethered Platinum Coils

All procedures were approved by the University of Pennsylvania Regulatory Affairs Committee. Sprague-Dawley rats (weight, 375 to 425 g) were given access to food and water ad libitum before anesthesia was induced by an intraperitoneal injection of 60 mg/kg sodium pentobarbital. After induction of anesthesia, the animals were kept on a heating pad and maintained at 37°C for the entire procedure and immediate recovery period. In the supine position, a right paramedian neck incision was made, exposing the CCA. The CCA was skeletonized, and then a permanent ligature was placed proximal to the CCA bifurcation, while a temporary ligature was placed 1 cm distal to the origin of the CCA (Figure 1A). After proximal control of the CCA was obtained with the temporary ligature, a small arteriotomy was made 2 mm proximal to the distal ligature. The coil segment coated with Ad-GFP was then inserted into the CCA, and a new ligature was placed just distal to the arteriotomy to exclude it from the circulation (Figure 1B). The temporary ligature was released to reestablish blood flow in the carotid segment. The operative field was inspected to ensure that the final ligature maintained hemostasis and that the coil was secure in the newly created blind-ended sac (Figure 1B). The wound was closed with 4-0 nylon sutures, and the animals were returned to their cages and allowed to recover for 7 days until vessel harvest. No studies were performed with the use of coils with only covalently attached antibody but without adenovirus.

Vessel and Coil Harvest

Animals were given access to food and water ad libitum, and then the coils were harvested from the CCA. Anesthesia was induced by an intraperitoneal injection of 60 mg/kg sodium pentobarbital, and the animals were killed by an intracardiac injection of 60 mg/kg sodium pentobarbital. The previous incision was opened, and the CCA was exposed. The segment of CCA containing the coil was removed. The section of CCA, approximately 10 mm in length, was sectioned in half with microscissors, and the coil was carefully removed. The coil and CCA segments were placed into 1× PBS.

Morphology Assessment

For the *in vitro* assessment, coil segments were removed from cell culture and evaluated for either green fluorescence (GDC) according to fluorescence microscopy or β -galactosidase expression (biodegradable coils) with the use of X-gal staining. Biodegradable GDCs were only used *in vitro* and only with β -galactosidase reporter constructs. For the *in vivo* assessment (GFP only), representative CCA segments were embedded in frozen section media (O.C.T., Tissue-Tek) and sectioned (5 μ m) for fluorescent and light microscopy examination. The retrieved coils were examined in a fresh state for the presence of GFP fluorescence.

Light microscopy studies used hematoxylin-eosin staining for general morphology and immunoperoxidase staining for detection of white blood cells (leukocyte common antigen), endothelial cells (von Willebrand factor), and fibroblasts (vimentin); the same immunohistochemistry techniques were used to confirm GFP expression. Briefly, sections were protein blocked with 10% normal horse serum (Vector) and then incubated overnight at 4°C with primary antibody. The following day, sections were incubated with anti-mouse biotinylated secondary antibody from the Vectastain Elite ABC kit (Vector). Vectastain avidin-biotin complex (Vector) was used to react with 3,3'-diaminobenzidine (DAB) peroxidase substrate (Vector) to produce a brown antigen staining. Immunohistochemistry studies were used to confirm expression of GFP by leukocytes. Frozen sections were first fixed with acetone. This was followed by a brief blocking with 10% normal horse serum and incubation with the first primary antibody for GFP. Incubation with a rat-adsorbed anti-mouse biotinylated secondary antibody (Vector) followed. Fluorescein avidin D (Vector) was then applied to the sections for 30 minutes. An avidin/biotin blocking kit (Vector) was used between primary antibody applications to prevent nonspecific binding. Sections were blocked again and then incubated in the second primary antibody, leukocyte common antigen, for 1 hour at 37°C. This was followed by application of the anti-mouse biotinylated secondary antibody. Sections were then treated with Texas Red Avidin D (Vector) for 30 minutes. Fluorescent mounting medium with DAPI was used for visualization of the cell nuclei. For fluorescence observations, frozen sections were fixed with 4% paraformaldehyde and mounted with DAPI for nuclei staining. GFP autofluorescence was quenched by a brief incubation in a 0.1% solution of Evan's Blue (J.T. Baker) and sodium borohydride (Aldrich) before cover slip placement. Microscopic pictures were taken on a Nikon Eclipse E800 epifluorescence microscope (Nikon Inc) equipped with SPOT version 3.02 software (SPOT Diagnostic Instruments) with the use of both fluorescein isothiocyanate (FITC) and DAPI filters. The number of GFP-expressing cells was determined by visual count in the arterial luminal spaces of $\times 200$ fields. Cell counts were performed by a single experienced observer, counting 3 fields in 3 separate sections for each artery. The total number of luminal cells counted per field was 231 ± 38 . Data were expressed as mean \pm SEM. The total number of cells in the same region was counted per DAPI-positive nuclei with the use of the nuclear counting macro of NIH Image version 1.62.

Polymerase Chain Reaction Studies of Biodistribution

Samples of tissues harvested for polymerase chain reaction (PCR) analyses were immediately frozen in liquid nitrogen. Phenol/chloroform DNA extraction was performed, and PCR amplification was performed over 35 cycles with the use of GFP-specific primers (upstream: 5'-GGC TGC TGC AAA ACA GAT AC-3'; downstream: 5'-CGG ATC CTC TAG AGT CGA C-3'). Amplified samples were analyzed with agarose gel electrophoresis with the use of appropriate standards, Ad-GFP, and a positive control.

Results

Platinum Coils In Vitro

When Ad-GFP–linked, collagen-coated platinum coils segments were placed in culture with rat A10 cells, an intense

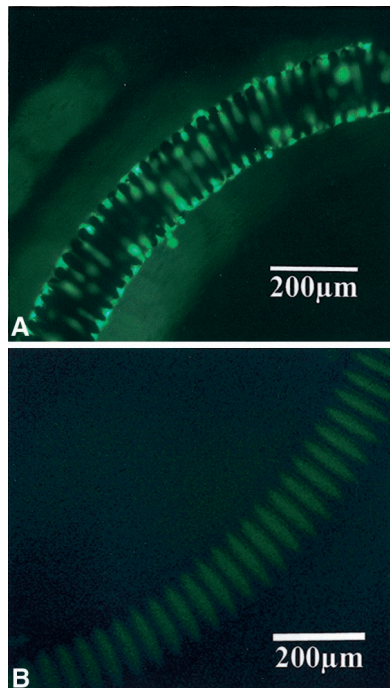


Figure 2. Site-specific Ad-GFP transduction using collagen-coated GDC with tethered vectors in cell culture (rat arterial smooth muscle, A10) demonstrated GFP-expressing cells exclusively on the coil segment and not in the culture. A, Positive transduction in vitro using collagen-coated platinum coils with tethered Ad-GFP (fluorescence micrograph, FITC filter). B, Negative control coil without modification.

and localized transduction of the cells growing on the coil surface occurred (Figure 2A). There was no cell-associated fluorescence on the unmodified platinum coil (Figure 2B). The underlying A10 cells growing on the bottom of the well displayed essentially no transduction in comparison to the widespread, uniform, and highly localized transduction observed with the cells growing on the coil surface, demonstrating a marked localization of transduction at the surface of the coil.

Biodegradable Coils In Vitro

When Ad-LacZ–linked, collagen-coated polyglycolide coils were cultured with rat A10 cells, transduction of cells was noted on 4 of 4 polyglycolide coils tested, as indicated by the blue cell-localized staining on their coil surface (Figure 3A). Additionally, there is evidence of a positive transduction on the surface of the biodegradable polyglycolide coil itself as well as within the collagen coating (Figure 3B). No X-gal staining was noted on other cells growing in the plate, nor was there positive staining in cultures with the use of biodegradable polyglycolide coils without tethered adenovirus (Figure 3C). There was qualitatively less transduction noted with the polyglycolide coil compared with the platinum coil (GFP results), perhaps because of differences in local chemistry at the site of the hydrolyzing polyglycolic acid or differences in reporter sensitivity.

In Vivo Coil-Based Gene Delivery

A total of 6 arterial segments with GDCs plus collagen/Ad-GFP were harvested. Coils were implanted for 7 days to

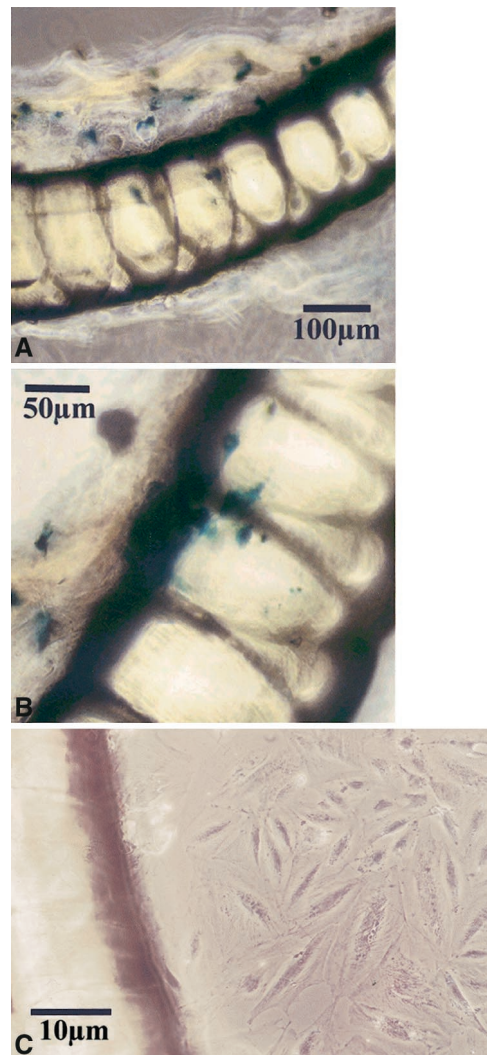


Figure 3. Positive transduction with biodegradable polyglycolide coil delivery of antibody-tethered Ad-LacZ onto the surface. A, Positive transduction (X-gal staining) on the surface of biodegradable polyglycolide coil itself and in the collagen coating. B, High-power view of positive transduction on the surface of the biodegradable polyglycolide coil. C, Control coil (no Ad-GFP) incubated with A10 cells and stained with X-gal (as above) showing no positive cells.

obtain GFP expression at its peak. In other studies with platinum coils alone,¹⁵ most changes in the histology, such as wall thickening, occur within 1 week, with less subsequent change in the following week. Harvested platinum microcoils were easily removed from the vessel and were seen by gross inspection to have minimal fibrotic encapsulation of the coil after 1 week of implantation.

Coil segments removed from the CCA were evaluated for transduction activity on the coil surface. CCA segments were sectioned and evaluated with DAPI staining for the cell nuclei and green fluorescence for GFP transduction (Figure 4). These segments showed widespread transduction within the intraluminal organized thrombus but not within the arterial wall (Figure 4A and 4B). Morphometric analyses revealed that 13.3±2.0% of cells within the organized thrombus were transduced with Ad-GFP via the gene delivery system; GFP

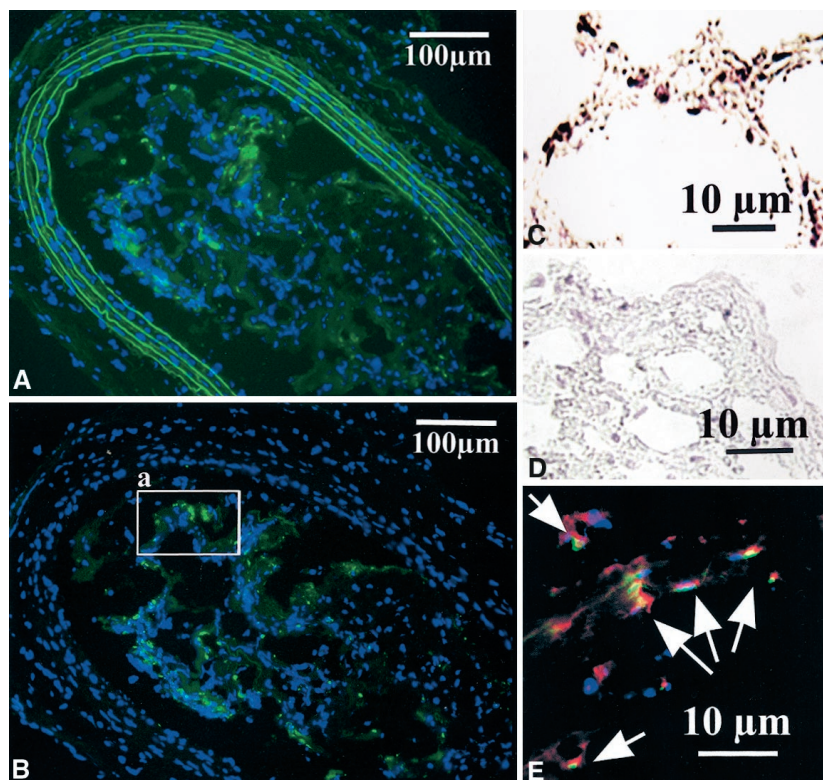


Figure 4. Cross section of CCA segments after 7-day implantation of Ad-GFP-tethered coil. A, An example of FITC (green fluorescence) and DAPI (blue fluorescence) merged photograph shows the cellular distribution of the GFP (green fluorescence) transduced cells within the lumen of the CCA and green fluorescence of arterial elastic laminae without cellular fluorescence (FITC and DAPI filters). B, Evans blue pretreatment subtracts autofluorescence of the arterial elastic laminae (vs A), highlighting the GFP fluorescence concentrated in the organizing thrombus in the arterial lumen; "a" corresponds to sections shown in C, D, and E (FITC and DAPI filters). C, Immunostaining with anti-GFP antibody (DAB, staining brown) demonstrates GFP expression within the organizing thrombus (B, region a), which is most intense in cells but is also present extracellularly. D, Immunostaining with a nonspecific antibody (DAB) reveals no nonspecific DAB-positive regions (an absence of brown coloration) within organizing thrombus (B, region a). E, Immunofluorescent staining for leukocyte common antigen (anti-leukocyte common antigen is rhodamine labeled, with red fluorescence) demonstrates (as indicated by arrows) colocalization of leukocyte common antigen, with DAPI (blue fluorescence) indicating nuclei and green fluorescence indicating GFP, as in C (from B, region a).

transduction was confirmed with immunohistochemistry (Figure 4C). The hematoxylin-eosin staining morphology demonstrated intraluminal organized thrombus (results not shown), with white blood cell invasion as confirmed by positive leukocyte common antigen staining (Figure 4E). Virtually all of the transduced cells were leukocytes (Figure 4E). There was no evidence of smooth muscle cell or endothelial cell transduction according to comparisons of fluorescence micrographs with serial hematoxylin-eosin cross sections and immunohistochemistry. Furthermore, there was no evidence of endothelialization of thrombi or fibroblast invasion in these 1-week explants according to immunohistochemistry studies (data not shown). Examination of explanted coils from the rats after 7 days demonstrated GFP-positive cells adherent to the coil surface (Figure 5), consistent with the cell culture results.

Bioavailability

Vector biodistribution was assessed with PCR studies of distal organs. Representative lung, liver, and kidney samples were evaluated from each animal by PCR analyses. There were no detectable GFP DNA sequences found after 35 cycles in any of the tissues sampled. This indicated that the virus biodistribution was localized to the site of deployment of the endovascular microcoil gene delivery system.

Discussion

These investigations are the first report of the incorporation of a gene delivery system onto the surface of an endovascular coil. Adenovirus was successfully tethered with antiviral antibody onto the surface of platinum microcoils with efficient gene delivery both in vitro and in vivo. The in vitro

results showed that the antibody was capable of localizing the adenovirus to the surface of the platinum coil. Rat arterial smooth muscle cells (A10) were used as a convenient model system to investigate site-specific transduction via antibody-tethered adenovirus immobilized on a GDC. Ideally, transduction of leukocytes invading a model thrombus would have better represented a simulation of the in vivo environment; however, this was not practical. Nevertheless, the A10 cell cultures proved useful for initial studies of GDC-based adenovirus delivery. This was extrapolated to an in vivo model and again showed positive transduction from the platinum coil expressed in the cellular material found within the CCA. There was virtually no GFP transduction noted in the arterial wall; GFP expression was only noted in the cells of the organizing thrombus and the adjacent arterial endothelium. These results are consistent with the PCR data demonstrating no downstream vector DNA and overall confirm the site specificity and tight localization of antibody-tethered vectors. Moreover, we investigated in vitro coupling of adenovirus to a completely biodegradable coil, which may have therapeutic advantages for promoting long-term aneurysm stabilization without the persistence of a metal implant.

The mechanism of vector delivery in these GDC studies very likely involves processing of the intact adenovirus-antibody complex, with eventual detachment of the vector from the immunoglobulin at some time before gene expression. Previous research from our group¹⁶ has demonstrated in cell culture studies that antibody-tethered adenovirus is predominantly taken up by cells as an intact antibody-vector complex, with exclusion of antibody after 24 hours. Thus, cellular interactions must be involved in breaking down either the collagen matrix or the poly(D,L-lactic-co-glycolic acid)

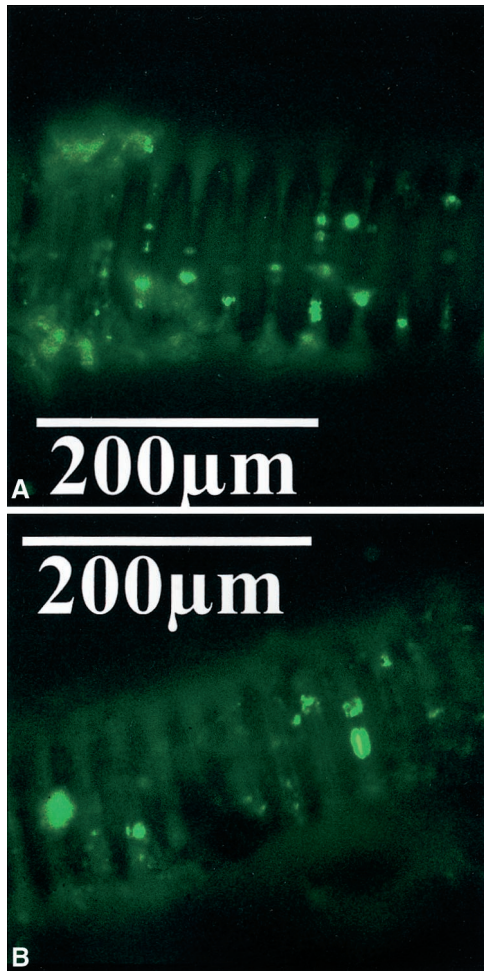


Figure 5. Representative platinum microcoils with antibody-tethered GFP adenoviral delivery system, retrieved from different rats after 7 days of implantation, removed from CCA segment, show tissue ingrowth and GFP-positive transduction throughout the coils (fluorescence micrograph, FITC filter).

(PLGA) to permit antibody detachment and cellular internalization. Furthermore, the absence of systemic distribution of vector in the rat studies, at the detection limits of PCR, also supports the view that the high degree of site specificity noted is due to local processing of covalently bound antibody, with tethered vector, rather than dissociation of vector from the antibody. A comparable result was seen in our pig coronary stent studies,¹⁶ in which we used Ad-GFP that was antibody tethered onto collagen-coated stents. Retrieval analyses in these coronary studies demonstrated GFP expression only in coronary sites in contact with stents, with no downstream coronary or distal organ expression. In the present studies our animal model is based on a blind-ended, high-pressure, arterial sac, which is quite different from the pathophysiological environment of the coronary artery after stenting. The objective of endovascular coil gene therapy is vascular occlusion of the target structure as opposed to vessel patency with coronary artery stent modifications. Thus, the implications of this work represent a whole new treatment armamentarium for cerebrovascular disease and perhaps even intracranial neoplasms.

Although a large number of intracranial aneurysms are currently treated with endovascular coil occlusion, little is known in terms of long-term effectiveness with respect to complete occlusion. In fact, there have only been anecdotal reports of a mere 26 aneurysms, which is a considerably small number of reported necropsy findings compared with the 10 000 to 15 000 aneurysms that have been coiled to date worldwide.^{6,18–23} There have been 6 human case reports of 8 aneurysms,^{18–23} and the largest single series has reported 18 aneurysms⁶ with documented histopathological results after GDC therapy. Overall, there was evidence of a thin membrane over the neck of the ostium of the aneurysm as early as 3 to 7 days and fibrous tissue as early as 1 month; endothelium covering the neck was not observed until 12 months. However, the absolute presence of peripheral fibrosis does not determine whether there is endothelium over the ostium, which is a necessity for complete aneurysm obliteration. Biologically active coils with gene or protein delivery capability can play a significant role in improving occlusion rates.

Previous experimental studies of models of intracranial aneurysms typically focused on the use of venous sidewall aneurysms of the CCA in which a segment of jugular vein was used.¹² These models are very costly, are difficult to establish, and use venous rather than arterial vessels for the aneurysm itself. Thus, our approach was to focus on evaluating arterial pathobiology in response to gene therapy with the use of a simpler rat model based on a blind-ended arterial sac. Thus, our rat studies attempted only to create a model of thrombosis/stasis to mimic the desired outcome of coil occlusion. In addition, this was considered appropriate in this proof of concept with the goal of using larger animals with sidewall jugular aneurysms for the next phase of experimentation. Prior investigations have used *in vitro* and *in vivo* models to investigate surface modification of GDCs with extracellular matrix proteins,^{8,10,14,24,25} nonbiodegradable polymers,^{12,26} ionizing radiation,¹³ and coating of the coils with immortalized fibroblasts that secrete growth factors.^{10,11} Previous studies performed by our group demonstrated that rhVEGF added to the surface of GDCs would promote angiogenesis necessary to provide a biological scaffolding for subsequent fibrosis, especially in larger aneurysms. Using the same rat model as in this study, we evaluated CCA segments with unmodified GDC, GDC plus collagen, and GDC plus collagen/rhVEGF. Our results showed that those CCA segments with GDC plus collagen/rhVEGF had significantly more occlusive tissue with an overall enhanced vascular response.¹⁵ Although these results were very promising, we further postulated that for improved aneurysm stabilization, platinum could be replaced with a thrombogenic and biodegradable polymer such as polyglycolide. Compared with GDC alone, our biodegradable coil showed a significantly enhanced vascular response.¹⁷

Thus, we speculate that combining gene therapy with endovascular technology would serve as a very novel method of providing a multitude of therapeutic modifications to platinum and biodegradable microcoils. Adenovirus exhibits high gene transfer efficiency compared with other vectors and has been used extensively in cardiovascular gene therapy.^{27–32} In particular, it has been used with animal models to prevent

restenosis after balloon-induced arterial injury, vein graft intimal hyperplasia, arterial thrombosis, and transplantation vasculopathy.³³ Current methods of adenoviral delivery include direct injection (either intravenous, intra-arterial, or intramuscular), the use of balloon catheter surface, or the use of biodegradable microspheres. Gene transfer efficiency and its subsequent therapeutic effect are crucial to these methods, as well as precise control of dose and biodistribution. In addition, adenoviral-mediated growth factors would possibly allow for continued release mechanisms compared with purely recombinant growth factors.

Conclusion

This is the first demonstration an endovascular microcoil that also functions as a gene delivery system. The next generation of coil technology emerges to incorporate a host of growth factors for aneurysm occlusion across those with larger domes and wider necks. Catheter deployment of gene delivery endovascular microcoils represents an interventional device-based gene delivery system that can serve as a suitable platform for either single or multiple gene therapy vectors. This approach has potential applications for improved treatment of intracranial aneurysms, arteriovenous malformations, and site-specific gene administration for end-organ therapy.

Acknowledgments

The authors thank Jennifer LeBold and Jeanne Connolly for their assistance in preparing the manuscript. This research was supported in part by National Institutes of Health grants HL41663 (Dr Levy) and HL64388 (Dr Diamond and Dr Levy), a grant from the Cystic Fibrosis Foundation, and the William J. Rashkind Endowment of the Children's Hospital of Philadelphia (Dr Levy).

References

- Debrun GM, Aletich VA, Kehrli P, Misra M, Ausman JI, Charbel F. Selection of cerebral aneurysms for treatment using Guglielmi detachable coils: the preliminary University of Illinois at Chicago experience. *Neurosurgery*. 1998;43:1281-1297.
- Gobin YP, Vinuela F, Gurian JH, Guglielmi G, Duckwiler GR, Massoud TF, Martin NA. Treatment of large and giant fusiform intracranial aneurysms with Guglielmi detachable coils. *J Neurosurg*. 1996;84:55-62.
- Richling B, Gruber A, Bavinski G, Killer M. GDC-system embolization for brain aneurysms: location and follow-up. *Acta Neurochir (Wien)*. 1995;134:177-183.
- Vinuela F, Duckwiler G, Mawad M. Guglielmi detachable coil embolization of acute intracranial aneurysm: perioperative anatomical and clinical outcome in 403 patients. *J Neurosurg*. 1997;86:475-482.
- Zubillaga AF, Guglielmi G, Vinuela F, Duckwiler GR. Endovascular occlusion of intracranial aneurysms with electrically detachable coils: correlation of aneurysm neck size and treatment results. *AJNR Am J Neuroradiol*. 1994;15:815-820.
- Bavinski G, Talazoglu V, Killer M, Richling B, Gruber A, Gross C, Plenck H. Gross and microscopic histopathological findings in aneurysms of the human brain treated with Guglielmi detachable coils. *J Neurosurg*. 1999;91:284-293.
- Abrahams JM, Diamond SL, Hurst RW, Zager EL, Grady MS. Topic review: surface modifications of Guglielmi detachable coils. *Surg Neurol*. 2000;54:44-51.
- Dawson RC, Krishit AF, Barrow DL, Joseph GJ, Shengelaia GC, Bonner G. Treatment of experimental aneurysms using collagen-coated microcoils. *Neurosurgery*. 1995;36:133-140.
- Dawson RC, Shengelaia GG, Krishit AF, Bonner GD. Histologic effects of collagen-filled interlocking detachable coils in the ablation of experimental aneurysms in swine. *AJNR Am J Neuroradiol*. 1996;17:853-858.
- Kallmes DF, Borland MK, Altes TA, Dion JE, Jensen ME, Hankins GR, Helm GA. In vitro proliferation and adhesion of basic fibroblast growth factor-producing fibroblasts on platinum coils. *Radiology*. 1998;206:237-243.
- Kallmes DF, Williams AD, Cloft HJ, Lopez MBS, Hankins GR, Helm GA. Platinum coil-mediated implantation of growth factor-secreting endovascular tissue grafts: an in vivo study. *Radiology*. 1998;207:519-523.
- Kwan ESK, Heilman CB, Roth PA. Endoscopic packing of carotid bifurcation aneurysms with polyester fiber-coated platinum coils in a rabbit model. *AJNR Am J Neuroradiol*. 1993;14:323-333.
- Murayama Y, Fernando V, Suzuki Y, Do HM, Massoud TF, Guglielmi G, Ji C, Iwaki M, Kusakabe M, Kamino M, Abe T. Ion implantation and protein coating of detachable coils for endovascular treatment of cerebral aneurysms: concepts and preliminary results in swine models. *Neurosurgery*. 1997;40:1233-1244.
- Szikora I, Wakhloo AK, Guterman LR, Chavis TD, Dawson RC, Hergenrother RW, Twyford RH, Hopkins LN. Initial experience with collagen-filled Guglielmi detachable coils for endovascular treatment of experimental aneurysms. *AJNR Am J Neuroradiol*. 1997;18:667-672.
- Abrahams JM, Forman MF, Grady MS, Diamond SL. Delivery of human vascular endothelial growth factor with platinum coils enhances wall thickening and coil impregnation in a rat aneurysm model. *AJNR Am J Neuroradiol*. 2001;22:1410-1417.
- Klugherz BD, Cunnixian S, Hinson JT, DeFelice S, Cui X, Lu Z, Connolly J, Wilensky RL, Levy RJ. Gene delivery to pig coronary arteries from stents carrying antibody-tethered adenovirus. *Hum Gene Ther*. 2002;13:443-454.
- Abrahams JM, Forman MS, Grady MS, Diamond SL. Biodegradable polyglycolide endovascular coils promote wall thickening and drug delivery in a rat aneurysm model. *Neurosurgery*. 2001;49:1187-1193.
- Castro E, Fortea F, Villoria F, Lacruz C, Ferreras B, Carrillo R. Long-term histopathologic findings in two cerebral aneurysms embolized with Guglielmi detachable coils. *AJNR Am J Neuroradiol*. 1999;20:549-552.
- Horowitz MB, Purdy PD, Burns D, Bellotto D. Scanning electron microscopic findings in a basilar tip aneurysm embolized with Guglielmi detachable coils. *AJNR Am J Neuroradiol*. 1997;18:688-690.
- Molyneux AJ, Ellision DW, Morris J, Byrne JV. Histological findings in giant aneurysms treated with Guglielmi detachable coils: a report of two cases with autopsy correlation. *J Neurosurg*. 1995;83:129-132.
- Mizoi K, Yoshimoto T, Takahashi A, Nagamine Y. Pitfall in the surgery of a recurrent aneurysm after coil embolization and its histological observation: technical case report. *Neurosurgery*. 1996;39:65-69.
- Shimizu S, Kurata A, Takano M, Hiroshi T, Yamazaki H, Miyasaka Y, Fujii K. Tissue response of a small saccular aneurysm after incomplete occlusion with a Guglielmi detachable coil. *AJNR Am J Neuroradiol*. 1999;20:546-548.
- Stiver SI, Porter PJ, Willinsky RA, Wallace MC. Acute human histopathology of an intracranial aneurysm treated using Guglielmi detachable coils: case report and review of the literature. *Neurosurgery*. 1998;43:1203-1205.
- Tamatani S, Ozawa T, Minakawa T, Takeuchi S, Kioke T, Tanaka R. Histologic interaction of cultured endothelial cells and endovascular embolic materials coated with extracellular matrix. *J Neurosurg*. 1997;86:109-112.
- Tamatani S, Ozawa T, Minakawa T, Takeuchi S, Kioke T, Tanaka R. Radiologic and histopathologic evaluation of canine artery occlusion after collagen-coated platinum microcoil delivery. *AJNR Am J Neuroradiol*. 1999;20:541-545.
- Ahuja AA, Hergenrother RW, Strother CM, Rappe AA, Cooper SL, Graves VB. Platinum coil coatings to increase thrombogenicity: a preliminary study in rabbits. *AJNR Am J Neuroradiol*. 1993;14:794-798.
- Barr E, Carroll J, Kalynch AM, Tripathy SK, Kozarsky K, Wilson JM, Leiden JM. Efficient catheter-mediated gene transfer into the heart and lung using replicant-defective adenovirus. *Gene Ther*. 1994;1:51-58.
- Chen SJ, Wilson SJ, Muller DWM. Adenovirus-mediated gene transfer of soluble vascular cell adhesion molecule to porcine interposition vein grafts. *Circulation*. 1994;89:1922-1928.
- Guzman RJ, Lemarchand P, Crystal RG, Epstein SE, Finkel T. Efficient and selective adenovirus-mediated gene transfer into vascular neointima. *Circulation*. 1993;88:2838-2848.
- Lafont A, Loirand G, Pacaud P, Vilde F, Lemarchand P, Escande D. Vasomotor dysfunction early after exposure of normal rabbits arteries to an adenoviral vector. *Hum Gene Ther*. 1997;8:1033-1040.
- Nabel EG. Gene therapy for cardiovascular disease. *Circulation*. 1995;91:541-548.
- Schneider MD, French BA. The advent of adenovirus: gene therapy for cardiovascular disease. *Circulation*. 1993;88:1937-1942.
- Kibbe MR, Billiar TR, Tzeng E. Gene therapy and vascular disease. *Adv Pharmacol*. 1999;46:85-150.