Use of the Rabbit Ear Artery to Serially Assess Foreign Protein Secretion After Site-Specific Arterial Gene Transfer In Vivo
Evidence That Anatomic Identification of Successful Gene Transfer May Underestimate the Potential Magnitude of Transgene Expression

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Background The development of molecular strategies for the treatment of restenosis has been hindered by low efficiencies of in vivo arterial transfection. Expression of intracellular marker proteins is generally evident in <1% of vascular smooth muscle cells after in vivo arterial transfection. Efforts to improve the efficiency of in vivo gene transfer have been further impeded by the use of transgenes encoding for intracellular marker proteins, necessitating tissue removal and limiting survey to one point in time.

Methods and Results To study gene expression on a serial basis in vivo and determine the relation between a secreted gene product and transfection efficiency after in vivo arterial gene transfer, a method for performing and serially monitoring gene expression in vivo was developed using the central artery of the rabbit ear. Liposome-mediated transduction of plasmid DNA containing the gene for human growth hormone (hGH) was successfully performed in 18 of 23 arteries. Serum hGH levels measured 5 days after transfection ranged from 0.1 to 3.8 ng/mL (mean, 0.97 ng/mL); in contrast, serum drawn from the control arteries demonstrated no evidence of hGH production. Serial measurement of hGH from transfected arteries demonstrated maximum hGH secretion 5 days after transfection and no detectable hormone after 20 days. Despite these levels of secreted gene product documented in vivo, immunohistochemical staining of sections taken from the rabbit ear artery at necropsy disclosed only rare cells in which there was evidence of successful transfection.

Conclusions These experiments demonstrate a useful method of performing serial in vivo analyses of gene expression after vascular transfection and that anatomic analyses of transfection efficiency may underestimate the potential magnitude of expression in the case of a secreted gene product. These findings have implications for the clinical application of somatic gene therapy because low-efficiency transfection with a gene encoding for a secreted protein may achieve therapeutic effects not realized by transfection with genes encoding for proteins that remain intracellular. (Circulation. 1994;89:785-792.)

Key Words • stenosis • transfection • genetics

Gene therapy, by virtue of its putative ability to alter at the cellular level those processes considered essential for tissue repair and proliferation of vascular smooth muscle cells, has been proposed as a therapeutic strategy to prevent restenosis. Although a variety of issues must be resolved for somatic gene therapy to be used in this fashion, none is more basic than the need to accomplish effective delivery of DNA to that segment of the arterial wall at which the proposed therapeutic effect is required.

Direct in vivo arterial gene transfer has been successfully performed with a variety of reporter genes, all of which required that the experimental animal be killed and necropsy examination be performed to identify evidence of reporter protein production. Furthermore, although the ability to transfer foreign DNA to the arterial wall in vivo has thus been convincingly demonstrated, nearly all attempts to perform arterial gene transfer have been complicated by a low transfection efficiency.

The precise reason for the low efficiency of transfection in previous studies may relate to a variety of factors, including the specific plasmid, vector, or delivery instrument used. Because previous reports of arterial gene transfer have relied principally on transfection with DNA encoding for intracellular marker proteins, however, opportunities to study and modify techniques intended to enhance transfection efficiency have been limited due to the need for tissue removal to document protein production. Assay for proteins such as β-galactosidase or luciferase, for example, necessitates removal of the transfected tissue and therefore limits the evaluation of transfection to one point in time. For in vivo transfection to become a viable method of implementing gene therapy...
for vascular diseases, the ability to serially monitor transfection efficiency in live animals would be of value.

The use of a secreted reporter protein is a logical alternative to intracellular markers and their associated need for tissue retrieval. Successful in vivo arterial transfection with subsequent identification in vivo of a secreted protein would permit serial evaluations of vascular transfection to be accomplished by using samples of serum to assay for the secreted protein. By avoiding the need for tissue retrieval, this method would permit serial evaluation of transfection in the same subject and thereby facilitate efforts to study strategies designed to enhance the magnitude, duration, or both of transfection efficiency. For each transfected artery, a series of data points would replace the single time point typical of transfection performed using intracellular reporter genes. In vivo detection of protein secretion after in vivo arterial gene transfer, however, has not been previously reported. This may be due in part to the relatively low efficiencies currently achieved by in vivo transfection in conventional animal models: correspondingly low-level production of the secreted protein from a remote site (eg, iliac or coronary artery) might result in nondetectable levels when the marker protein is diluted in the total blood volume of the experimental animal.

Nabel et al demonstrate that local biological effects result from in vivo transfection with transgenes encoding for proteins that are either expressed at the cell surface or secreted. From a practical standpoint, biological effects limited to the site of secretion might be desirable in some cases. Favorable and important regional effects, for example, might be achieved by prevention of intra-arterial thrombus formation or inhibition of vasoconstriction at the site of balloon angioplasty; unnecessary systemic effects, including systemic thrombolysis and vasodilatation, respectively, would be avoided in such cases.

To solve the problem of detecting low-level protein secretion after in vivo vascular transfection, we designed a technique that uses the central artery of the rabbit ear. By isolating the circulation of the ear during blood sampling, the dilutional effect of continuous blood flow through the transfected vessel segment is eliminated. We anticipated that this method would permit the detection of locally secreted protein in amounts that may not be evident systemically. This technique confers other potential advantages in the evaluation of intravascular protein secretion after in vivo transfection. The central artery of the rabbit ear is superficial and therefore easily accessible for transfection and later blood sampling. The technical ease of access facilitates serial evaluations that might complicate sampling from sites used in other reports of in vivo transfections, such as the iliac and coronary arteries. In the rabbit ear, these serial manipulations can be performed with minimal risk of attrition, thus providing the opportunity for longitudinal follow-up in a single subject. Finally, tissue analysis performed at necropsy affords the opportunity to study the relation between anatomically evident transfection and the magnitude of gene expression in vivo. Accordingly, the present investigation used the rabbit ear artery to (1) document arterial gene transfer in vivo, (2) serially monitor the time course of the resulting expression during life, and (3) determine the corresponding anatomic extent of vascular transfection responsible for a given level of antemortem gene expression (ie, protein production).

Methods

Luciferase

Preliminary investigation using the rabbit ear central artery for liposome-mediated arterial gene transfer was undertaken using an intracellular marker protein previously used for arterial gene transfer. The intent of this phase of the study was to determine with a transgene established for use in arterial transfection the potential for in vivo transfection using the central artery of the rabbit ear and, with this technique, to establish the site of transfection within the target vessel segment. The central artery was transfected with a plasmid (pRSVLUC) consisting of a full-length firefly *Photinus pyralis* luciferase cDNA (pJD 204) under the control of the Rous sarcoma virus long terminal repeat promoter (courtesy of Dr Allen Brasier), which was inserted into a plasmid derived from pGEM3 (Promega, Madison, Wis).

In these preliminary studies, a total of five New Zealand White rabbits (NZWs) were sedated with an intramuscular injection of xylazine (5 mg/kg) and then anesthetized by intramuscular injection of a combination of ketamine and acepromazine (10 mg/mL ketamine plus 1.5 mg/mL acepromazine at a dose of 0.5 mL/kg). The central artery of the NZW ear was isolated by applying a tourniquet to the base of the ear (Fig 1), temporarily interrupting blood flow to the ear and thereby providing a stable environment for vascular transfection. The artery was then cannulated with a 23-gauge needle, and serum-free medium (Opti-MEM, Gibco, Gaithersburg, Md) was infused until all visible blood had been cleared from the target segment. The superficial nature of the central artery allowed for visual confirmation that blood had been successfully cleared. A solution of liposomes and plasmid DNA (pRSVLUC) was then infused into the isolated segment and incubated for 30 minutes. The DNA-liposome solution was prepared no more than 15 minutes before the transfection procedure. A total of 30 μg of DNA and 90 μg of cationic liposomes (N-[1-(2,3-dioleoyloxy)propyl]N,N,N-trimethylammonium chloride, Lipofectin, BRL, Gaithersburg, Md) was added to the serum-free medium (Opti-MEM, Gibco) to a final volume of 1 mL at room temperature in a sterile polystyrene tube. The solution was then drawn into a sterile syringe and injected into the artery through the indwelling 23-gauge needle. The solution used in the present series of experiments consisted of plasmid DNA (30 μg) and liposome (90 μg) at a ratio of 1:3 in a total volume of 1 mL of serum-free medium. This ratio was previously reported to optimize transfection efficiency in cultures of vascular endothelial cells.

The arteries were harvested 5 days later and assayed for evidence of luciferase activity. Monitoring of luciferase activity was done using the luciferase assay system (Promega) as previously reported. Briefly, the artery to be analyzed was suspended in 1× cell culture lysis reagent and homogenized (Virtis, Gardiner, NY). The resulting mixture was spun for 5 minutes to pellet large debris. A 20-μL aliquot of the cell extract was then mixed in a sample tube with 100 μL of luciferase assay reagent at room temperature and inserted into a luminometer (model 20e, Turner Design, Sunnyvale, Calif). Three different aliquots for each specimen were analyzed in this manner. The specimen’s total luciferase activity was derived from the mean of the aliquot results. The light unit values obtained were within the linear range of a dilution curve established by analysis of light units produced from a known amount of luciferase (Sigma Chemical Co, St Louis, Mo). Although assays of different dilutions of luciferase standards constantly yielded linear curves, conversion into pico-
grams resulted in important level variations depending on the source of the standard, as others have reported. Thus, results are expressed as Turner light units (TLU). As a reference, under our conditions, transfection of rabbit vascular smooth muscle cells in culture (80% confluent) in 60-mm dishes for 30 minutes using 15 μg of pRSVLUC and 45 μg of Lipofectin yielded 431.25±49.13 TLU per dish (total from all cells [n=5]) when assayed 3 days later. Background level was always measured by analyzing a sample consisting only of cell culture lysis reagent and luciferase assay reagent but lacking homogenized tissue; all such analyses consistently produced readings of 0 TLU.

Human Growth Hormone

To determine if liposome-mediated in vivo arterial transfection could produce detectable local levels of a secreted protein, we used a similar protocol for transfection with a plasmid (pXGH5) containing the human growth hormone (hGH) gene driven by the mouse metallothionein (mMT) promoter. This gene was chosen because hGH is a secreted protein that can be easily assayed and has been expressed in vivo after in vitro transfection.

A total of 15 NZWs were anesthetized, as described above. The central artery of the NZW ear was isolated by applying a tourniquet to the base of the ear (Fig 1). The artery was then cannulated with a 23-gauge needle inserted near the tip of the ear, and serum-free medium (Opti-MEM; GIBCO) was infused until all visible blood had been cleared from the target segment. A solution of liposome and plasmid DNA (pXGH5) was then infused into the isolated segment and incubated for 30 minutes. The DNA-liposome solution was prepared no more than 15 minutes before the transfection procedure. A total of 30 μg of DNA and 90 μg of cationic liposome (Lipofectin; BRL) was added to the serum-free medium (Opti-MEM; GIBCO) to a final volume of 1 mL at room temperature in a sterile polystyrene tube. The solution was then drawn into a sterile syringe and injected into the artery through the indwelling 23-gauge needle. After infusion of the DNA-liposome solution, the target segment of artery was further isolated by the application of clamps externally over the central artery proximal and distal to the transfected site. The solution was then allowed to incubate as a stagnant, clamped fluid column in the tourniquet ear for 30 minutes. After 30 minutes, the tourniquets and clamps were released, and the animals were allowed to recover.

In the first 7 animals, the right ear was transfected with the pXGH5 plasmid. The left ear was treated in identical fashion with tourniquet-induced blockade of blood flow, clearing of blood with serum-free medium, and infusion of liposome solution that was, however, devoid of plasmid DNA. In the remaining animals, both ears were transfected with plasmid DNA.

Because the mMT promoter in the pXGH5 plasmid is responsive to heavy metals such as cadmium and zinc, the drinking water of the first 5 rabbits was supplemented with zinc sulfate (70 mmol/L). However, when the serum zinc levels of the rabbits not receiving supplemental zinc were measured, they were found to be adequate to drive the promoter, based on previously reported in vitro evidence, and the practice of adding zinc to the rabbit diet was discontinued.

Human Growth Hormone Assay

Assays for hGH levels were performed on samples of blood drawn from the transfected segments of artery. In preparation for the assays, rabbits were anesthetized as described for the initial transfection procedure. The circulation of both ears was again isolated using a tourniquet applied to the base of the ear. After 10 minutes, each artery was cannulated with a 23-gauge needle, and blood was drawn until no further blood could be extracted. This resulted in the extraction of 0.5 to 1 mL of blood; in all cases, the amount of blood extracted was sufficient to perform duplicate radioimmunoassays for hGH. Blood samples used to assay for hGH were obtained at 2- to 7-day intervals until hGH levels were no longer detectable.

The assay used to detect hGH was a commercially available radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, Calif). Briefly, this assay uses two murine antibodies, each of which is specific for a different and distinct epitope on the hGH molecule. The antibodies bind without competition or steric interference. One antibody is radiolabeled with 125I, and the other is coupled to biotin. The addition of an avidin-coated bead to the reaction mixture provides a specific method of binding the avidin/antibody-hGH–radiolaied antibody complex to a solid phase for assay. A radioactivity-hGH concentration dose-response curve was generated from a series of standards with known hGH concentrations. Concentrations of hGH in samples of rabbit serum were derived directly from this curve. All standards and unknown samples of serum and culture
medium were assayed in duplicate. The lowest measurable concentration of hGH with this assay is 0.1 ng/mL. The sensitivity of the assay, defined as the smallest value that can be distinguished from zero at the 95% confidence limit, is 0.06 ng/mL.

Results of hGH concentration in serum and culture media are reported as mean±SEM. A correlation coefficient was calculated for each of the standard curves generated. The mean correlation coefficient of the standard curves generated using samples with known hGH concentrations was $R = 0.988 ± 0.019$.

**Immunohistochemistry**

A murine monoclonal antibody specific for hGH was used to identify cells responsible for the production of hGH. Immunostaining of arteries that had been successfully transfected in vivo would therefore permit identification of individual transfected cells and thus indicate the relation between the anatomic extent of cellular transfection observed at necropsy and transgene expression detected during life by serial radioimmunoassays. In alternate, alternate sections were stained with antibodies to smooth muscle $\alpha$-actin (HHF-35) and factor VIII–related antigen to identify vascular smooth muscle cells and endothelial cells, respectively, and thereby establish the identity of the transfected cells.

To identify at necropsy individual cells transfected in vivo, immunostaining was performed on transfected arteries that had demonstrated significant hGH production by radioimmunoassay. Assay for hGH production was performed immediately before removal of the artery to confirm the production of hGH and establish the level of hGH output. The animals were anesthetized, and blood was drawn for hGH assay as described above. The needle used for blood drawing was left in place in the artery while the animal was euthanized. The indwelling needle was then used to infuse a solution of acetone, methanol, or 2% formalin for fixation of the artery. The fixative was infused with the tourniquet applied to the base of the ear to keep the artery engorged during fixation. The artery was fixed in situ for 10 minutes and then dissected free and placed in an identical solution overnight, after which it was embedded in paraffin and sectioned. Sections were obtained at 1-mm intervals along the entire length of the transfected artery segments.

The immunostaining procedure was performed as follows. After deparaffinizing, sections were incubated in 3% hydrogen peroxide in water for 5 minutes and then rinsed in phosphate-buffered saline (PBS) for 5 minutes. Next, the primary antibody, an ascites-derived murine monoclonal antibody to hGH (clone 54/9 2A2; Biogenex, San Ramon, Calif), was applied and incubated at room temperature for 30 minutes. After rinsing in PBS, a linking antibody consisting of biotinylated immunoglobulins for mouse, rabbit, guinea pig, and rat was applied and allowed to incubate for 20 minutes at room temperature. The slides were rinsed again, after which the label, peroxidase-conjugated streptavidin, was applied; incubated for 20 minutes at room temperature; and rinsed in PBS. The substrate, 3,3′-diaminobenzidine tetrahydrochloride, was then added and incubated for 40 minutes at room temperature. Sections were lightly counterstained with Gill's hematoxylin.

**Immunostain for $\alpha$-Actin and Factor VIII**

Alternate sections were stained with antibodies to smooth muscle $\alpha$-actin (HHF-35; Enzo Diagnostics, Farmingdale, NY) and factor VIII–related antigen (Signet Labs, Dedham, Mass) according to the manufacturers' guidelines.

**Histology**

Conventional light microscopic examination of the central ear artery was performed in two animals 1 week after the transfection procedure. Both of these animals had undergone transfection as described above, and two subsequent blood samplings had been taken before they were killed. In each case, the artery was perfused with 2% formalin in situ before removal as described above. Once harvested, the transfected segment was serially sectioned at 2-mm intervals along the length of the transfected segment; alternate sections were stained with hematoxylin and eosin and trichrome-elastic stains.

**Statistical Analysis**

Results are expressed as mean±SEM. Statistical differences between transfected and control arteries were determined using a paired two-tailed Student's $t$ test. Differences were considered statistically significant if $P < 0.05$.

**Results**

**Luciferase Assay**

Percutaneous, site-specific arterial gene transfer with pRSVLUC was attempted in a total of 10 arteries. When these 10 arteries were assayed 5 days after the transfection procedure, 5 showed evidence of luciferase activity with a mean light production of 1.2±0.75 TLU. Only the transfected artery segment was the only possible source of the luciferase detected and thus established as the site of transfection. This preliminary evidence suggested that the technique designed for these studies was capable of successful site-specific arterial gene transfer.

**Human Growth Hormone Assay**

Transfection with the gene encoding for hGH was attempted in a total of 23 central ear artery segments in 15 rabbits; the remaining 7 ears were used as controls. Transfection was unsuccessful in rabbit 1 because of our inability to cannulate the artery; rabbit 5 died unexpectedly from an unknown cause 2 days after transfection. In all other cases, the rabbits survived the transfection procedure, as well as all subsequent procedures, without sequelae.

Transfection was successful as evidenced by hGH production in 18 of 21 arteries in which cannulation had been successfully accomplished and after which the animal survived ≥4 days. Serum samples from the 21 transfected arteries assayed on day 5 yielded 0.97±0.41 ng/mL hGH (range, 0.1 to 3.8 ng/mL hGH). In contrast, serum drawn from the control arteries yielded 0.01±0.01 ng/mL, a level below the sensitivity of the test$^{18}$ and therefore considered equal to 0 ($P < 0.02$). Protein continued to be detectable in the serum for as long as 20 days after transfection; levels from samples obtained on days 6 through 20 disclosed 0.36±0.14 ng/mL ($P < 0.03$ versus controls) ($\text{Fig} \; 2$); levels for the entire time (days 4 through 20) yielded 0.57±0.16 ng/mL ($P < 0.01$ versus control). The time course of hGH secretion therefore displayed maximum levels on day 5, followed by a significant decline. No hGH was detected more than 20 days after transfection. No differences in hGH production were noted between animals receiving supplemental zinc and those receiving no zinc.

Thus, hGH was locally detectable in the serum for nearly 3 weeks after in vivo transfection in this animal model. The absence of hGH from control arteries established that the transfection procedure itself did not result in a false-positive assay for hGH and documented that the systemic circulation, also represented by samples drawn from the control ears, contained insignificant
levels of hGH. Therefore, the hGH detected could be derived only from local transfection of the arterial wall. Circulating lymphocytes or other blood elements could not be the source of the hGH because samples obtained from the control ears consistently disclosed no hGH.

**Immunohistochemistry**

Immunohistochemical staining, using a monoclonal antibody specific for hGH, was performed to identify hGH production as an indicator of specific cells that had been successfully transfected. Despite biologically significant levels in hGH production, immunostaining of arteries transfected in vivo revealed only rare vascular smooth muscle cells in which there was positive staining at necropsy (Fig 3). Staining of alternate sections with monoclonal antibodies to smooth muscle α-actin (HHF-35) and factor VIII revealed that the transfected cells corresponded to those staining positively for HHF-35 and therefore were vascular smooth muscle cells.

**Histology**

Despite manipulation of the artery required for the primary transfection procedure and as many as four subsequent procedures performed to obtain blood for hGH assay from the central ear arteries in individual animals, all arteries remained patent and functional throughout the study. Histological examination of those arteries performed 1 week after transfection and two blood draws revealed no loss of arterial integrity (Fig 4). Examination of representative sections from the entire length of the transfected segment of sampled arteries revealed no evidence of arterial injury.

**Discussion**

The purpose of the present investigation was to establish a live animal model that would achieve the following objectives: (1) document site-specific arterial gene transfer in vivo, (2) serially monitor the time course of gene expression after arterial gene transfer in vivo, and (3) determine the relation between gene expression in vivo and anatomic evidence of transfection efficiency at necropsy.

These goals could not be realized in animal models previously used to accomplish site-specific arterial gene transfer because of specific limitations inherent in these models. First, expression of reporter genes, including lac-Z and firefly luciferase, used in previous animal models is characterized by protein products that are not secreted; the fact that such proteins remain intracellular requires that the animal be killed for the transfected sites to be harvested and assayed for evidence of successful gene transfer. Second, in vivo sampling from arteries, including coronary and peripheral, previously used for site-specific delivery can be complicated by dilution from systemic blood as well as the need for catheters designed to access the intravascular site.

The rabbit ear artery model we described circumvents these limitations. Gene expression is detected in vivo by assay for a secreted protein. Because the central ear artery is easily and repeatedly accessible, direct arterial transfection is readily achieved, and the local circulation may be interrupted repeatedly to sample for the resultant protein. Moreover, because commercially available assays are available to monitor hGH production in vivo and conventional immunostaining permits detection of the marker protein in histological sections harvested from the transfected segment of artery, gene expression in vivo may be related to the anatomic extent of gene transfer observed at necropsy.

Accordingly, the rabbit ear artery model allowed successful execution of each of the three objectives outlined above. Successful gene transfer was documented in vivo in 18 of 21 arteries in which cannulation had been successfully accomplished and in which the animal had survived ≥4 days. In 16 of the 18 arteries, the time course of gene expression was serially monitored and demonstrated maximum protein production at 5 days after transfection, after which there was a substantial decline in protein production until the animals were killed 20 days after transfection. No protein was detected more than 20 days after transfection.

Important distinctions exist between this model and previous models in which hGH has been used to evaluate the success of gene transfer. Previous models described by Barr and Leiden and Dhawan et al, used hGH as a reporter system to evaluate systemic delivery of recombinant proteins issued from an intramuscular depot. Gene transfer was achieved indirectly using retrovirus or other vectors to transf ect myoblasts in vitro before in vivo delivery. On intramuscular injection, the genetically modified myoblasts appear to fuse into adjacent normal muscle fibers, releasing the secreted protein product into the highly
vascular circulation characteristic of skeletal muscle. This approach has yielded systemic levels of hGH ranging from 0.28±0.08 ng/mL (using the pRSVGH plasmid) at 3 weeks\textsuperscript{16} to 16.1 ng/mL (using a retroviral vector) at 85 days after transfection.\textsuperscript{17}

The local levels of secreted protein achieved after direct gene transfer to the arterial wall in the present animal model (0.97±0.41 ng/mL at 5 days) compare favorably with the results cited above using a nonviral vector. Although numerous studies of normal hGH in human patients have shown a wide range of values, making a “normal” value difficult to define, the local levels of hGH achieved in the current series of experiments are within the range of physiological levels for normal human subjects.\textsuperscript{19} Immunohistochemical staining of the rabbit ear artery retrieved at necropsy accomplished the third objective of this study and indicated that the “physiological” levels of hGH achieved in this model were associated with only rare cells in which there was evidence of successful transfection.

The low number of cells transfected, as documented directly at necropsy, was anticipated based on previous experience with in vivo arterial transfection.\textsuperscript{2,3,5} In these previous experiments, which relied exclusively on the use of nonsecreted gene products, examination by histochemical staining,\textsuperscript{2,3} in situ hybridization,\textsuperscript{5} and/or polymerase chain reaction\textsuperscript{4} suggested that the transfection efficiency of direct gene transfer to vascular smooth muscle cells within the arterial wall was considerably less than 1% and therefore might preclude a meaningful biological response. In contrast, the present series of experiments illustrates that genes encoding for a secreted protein may overcome the handicap of inefficient transfection by a paracrine effect, secreting adequate protein to achieve local levels that may be physiologically meaningful. Nabel et al\textsuperscript{6} demonstrate that despite similarly low efficiencies, cell surface protein expression resulting from percutaneous transfection of vascular smooth muscle cells with the histocompatibility gene HLA-B7 may be adequate to induce a biological response, namely, focal vasculitis. Necropsy evidence of a pathobiological response following arterial gene transfer was reported by the same group, who used transgenes encoding for the secreted proteins PDGF-B\textsuperscript{7} and
FGF-1. It remains possible that the transfection efficiency for genes encoding secreted proteins may be further amplified by incorporation in an adenoviral vector, as recently reported for genes encoding nonsecreted marker proteins.

Secretion of protein after in vivo transfection has potential implications for the treatment of a variety of vascular disorders, particularly those that might benefit from secretion of thrombolytic proteins, angiogenic growth factors, or both. In these cases, the transfection procedure could be combined with a standard diagnostic or therapeutic intervention. For such applications of vascular gene therapy in humans, it will probably be necessary to modulate the transcription, translation, and secretion of recombinant proteins, as previously demonstrated in vitro. The rabbit ear model, by virtue of its ability to preserve the experimental subject through repeated assessments, is suitable for examining these regulatory mechanisms. Use of the central artery of the rabbit ear in this study permitted documentation of foreign protein secretion after in vivo transfection in quantities that could not be detected by sampling blood from the systemic circulation. This model therefore establishes the important advantage of enabling serial in vivo detection of the protein products resulting from transfected recombinant DNA in experiments designed to improve the efficiency and predictability of site-specific vascular gene therapy.

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