
Systemic Gene Therapy for Cardiovascular Disease

Katherine Parker Ponder

Systemic gene therapy involves the transfer into the body of a gene whose protein product reaches the blood and has a beneficial effect on a patient. Both retroviral and adenovirus-associated viral vectors have resulted in stable but only moderate systemic levels of blood proteins. Adenoviral vectors have resulted in very high levels of expression that diminishes over days or weeks. Hepatic gene therapy has achieved levels of the anti-coagulant protein C in blood that would protect against spontaneous thromboses in homozygous protein-C deficiency, and levels of tissue plasminogen activator that can lyse pulmonary emboli. Hypercholesterolemia has been ameliorated transiently by transfer of the low-density lipoprotein receptor gene into the livers of animals with familial hypercholesterolemia or by promoting lipid transfer via a variety of alternative mechanisms. Hypertension has been reduced by the transfer of genes for kallikrein or atrial natriuretic peptide into the liver, or by expressing antisense for the angiotensin II type 1 receptor after intravenous injection in neonates. Finally, fasting but not fed hyperglycemia has been ameliorated in animal models of diabetes by transfer of an insulin gene into the liver or by expression of insulin from implanted fibroblasts. Gene therapy has the potential to treat these cardiovascular diseases. However, improvements in levels of long-term expression and the ability to regulate expression in response to physiologic changes will be required before this approach will be implemented for most of these disorders in humans. (Trends Cardiovasc Med 1999;9:158-162). © 1999, Elsevier Science Inc.

Gene therapy involves the transfer of genetic information into a patient to correct a congenital or acquired disorder. The term "systemic gene therapy" refers to the in vivo production of a protein that reaches the blood, where it has ef-

fects throughout the body. Systemic gene therapy could treat thromboembolic disease, hypercholesterolemia, diabetes mellitus (DM), or hypertension, and will be the focus of this review. A summary of the genes that have been used to try to correct these disorders, and the efficacy of the various approaches, is given in Table 1. Localized gene therapy refers to the in vivo production of a protein that acts intracellularly or on a nearby cell. Although this approach holds great promise for the treatment of atherosclerosis by preventing restenosis after angioplasty or inducing angiogenesis to provide collateral blood flow (Vassalli and Dichek 1997, Nabel 1999), this topic will not be discussed here.

Katherine Parker Ponder is at the Departments of Internal Medicine and Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, Missouri, USA.

* Address correspondence to: Dr. Katherine Ponder, Box 8125, Division of Hematology, Department of Internal Medicine, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110, USA.

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• Basic Steps Required for Systemic Gene Therapy

Successful long-lived systemic gene therapy has three requirements: efficient gene transfer, appropriate expression, and long-term survival of transduced cells. Efficient transfer of the gene into a cell capable of producing a functional protein that can reach the blood must be achieved. Most recent studies have used an in vivo approach, where the gene is delivered directly to cells in the body via a viral vector (Robbins and Ghivizzani 1998, Wivel and Wilson 1998). Retroviral and adenovirus-associated viral (AAV) vectors can integrate into the host cell chromosome, but they are somewhat difficult to produce at the high concentrations needed for in vivo delivery. Most retroviral vectors are limited by their inability to transfer genes into nonreplicating cells, whereas the capacity of AAV vectors is only 4.5 kb or less. Adenoviral vectors have a large capacity, can be produced at very high concentrations, and can transfer genes into a variety of cell types regardless of their replication status. Their major disadvantage is their instability, which is due in large part to immunologic rejection of cells that express residual adenoviral genes, although the failure to integrate into the host chromosome may contribute.

Hepatocytes are an obvious target for systemic gene therapy, as they have direct contact with the blood. AAV (Snyder et al. 1997) and adenoviral (Kozarsky et al. 1994) vectors can transduce approximately 5% and 100% of nondividing hepatocytes, respectively. Moloney-based retroviral vectors result in transduction of 5%-10% hepatocytes (Rettinger et al. 1994), but they require induction of hepatocyte replication for efficient gene transfer. This can be achieved with hepatic growth factors, which have no or few adverse effects (Bosch et al. 1996, Patijn et al. 1998a, Gao et al. 1999), or with removal or damage to part of the liver (Rettinger et al. 1994; Patijn et al. 1998b), which is more invasive and therefore unattractive for use in humans. Retroviral vectors derived from lentiviruses can transfer genes into nondividing cells, but they appear to be inefficient at transducing hepatocytes (Kafri et al. 1997).

The muscle has been a popular target for in vivo delivery of adenoviral and AAV vectors because of its accessibility

Table 1. Summary of results of gene therapy experiments for cardiovascular diseases

<i>Disorder</i>	<i>Animal model</i>	<i>Vector used</i>	<i>Peak response</i>	<i>Duration</i>	<i>Clinical effect</i>
Thrombotic disease	Normal Rat	RV with PC to liver	Plasma PC 200 ng/mL (5% of nl)	>1 year	Not evaluable; should prevent most thrombosis
	t-PA (-) mice	Ad with tPA to liver	Serum t-PA >1 µg/mL (500-fold >nl)	1 week	Lyse 98% of PE
	PAI-1 + mice				Lyse 30% of PE
High cholesterol	LDL-R (-) rabbits	Ad with LDL-R to liver		2 weeks	TC fell from 900 to 250
		Ad with apo B mRNA editing enzyme to liver		4 weeks	TC fell from 650 to 450
		RV with LDL-R to liver		3 months	TC fell from 770 to 570
	LDL-R (-) mice fed a high cholesterol diet	Ad with LDL-R to liver		1 week	TC fell from 400 to 150
		Ad with VLDL-R to liver		2 weeks	TC fell from 400 to 200
		Ad with LPL to liver	Plasma LPL 80 mU/mL after heparin (7.5-fold >nl)	9 days	TC fell from 400 to 230
	LDL-R (-) humans	RV with LDL-R		1 year	TC fell from 671 to 608
Apo E (-) mice	Ad with apo E to liver	Serum apo E 0.2-5 mg/mL (10-fold >nl)	3 weeks	TC fell from 750 to 100; 64% ↓ in atherosclerotic lesion size	
	Ad with apo AI to liver	Apo AI ↑ from 1.1-2.4 mg/mL	1 month	HDL-chol ↑ from 60 to 174 mg/dL; 44% fall in atherosclerotic lesion size	
	Ad with LPL to liver	Plasma LPL 85 mU/mL after heparin	9 days	TC fell from 700 to 360	
Diabetes	Streptozocin-treated rodents	RV with insulin to liver or implanted fibroblasts	Serum insulin 7 ng/mL	Up to 4 weeks	Fasting glucose fell from 300 to 150 mg/dL; fed glucose, no improvement
Hypertension	Salt-sensitive and Goldblatt hypertensive rats	Ad with kallikrein to liver	Serum kallikrein levels 250 ng/mL	3 weeks	SBP fell from 190 to 175 mm Hg; ↓ cardiac hypertrophy and renal damage
		Ad with ANP to liver		3 weeks	SBP fell from 250 to 220 mm Hg; ↓ strokes, cardiac hypertrophy, and renal damage
	Spontaneous hypertensive rats	RV with AT-1 anti-sense intracardiac to neonates		4 months	SBP fell from 164 to 116 mm Hg; ↓ in cardiac hypertrophy

For animal models, (-) designates deficiency of a particular protein, and (+) designates overexpression. For the vector used, RV indicates retroviral vector, and Ad indicates adenoviral vector. The following abbreviations refer to the following therapeutic genes: PC, protein C; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor 1; LDL-R, low-density lipoprotein receptor; VLDL-R, very-low-density lipoprotein receptor; AT-1, angiotensin II type 1 receptor; LPL, lipoprotein lipase; and ANP, atrial natriuretic peptide. The following abbreviations refer to the following clinical effect: PE, pulmonary emboli; TC, total cholesterol; HDL-chol, high-density lipoprotein cholesterol; and SBP, systolic blood pressure.

to percutaneous injection (Herzog and High 1998). The majority of cells in a 1- to 2-mm region can be transduced (Kessler et al. 1996), making it necessary to perform multiple injections. Retroviral vector gene transfer has only been achieved using an ex vivo approach in which cells are removed, transduced in culture, and reimplanted (Herzog and High 1998), or by using lentiviral vectors (Kafri et al. 1997). Although some proteins, such as the 55-kD factor IX (Herzog and High

1998) and the 30-kD erythropoietin (Kessler et al. 1996), can readily reach the blood from muscle, others, such as the 68-kD β-glucuronidase, do not (Daly et al. 1999). Other targets for systemic gene therapy have included skin (Meng et al. 1998), fibroblasts attached to matrices (Chang et al. 1999), and bone marrow-derived cells (Cherington et al. 1998).

The second step for gene therapy is to achieve sufficient levels of expression to exert a clinical effect in the patient. This

has been achieved with many proteins using adenoviral vectors, but the effect generally disappears after days or weeks, and readministration is ineffective due to neutralizing antibodies. Long-term and clinically effective levels of expression have been achieved for several proteins that normally are present at moderate levels (5-10 µg/mL), such as factor IX (Herzog and High 1998, Synder et al. 1997), factor X (Le et al. 1997), or protein C (PC) (Cai et al. 1998), using retro-

viral or AAV vectors, delivery to either liver or muscle, and promoters that remain active in vivo. For proteins with potent biologic activities, expression needs to be regulated to avoid adverse effects such as the severe polycythemia that occurs in most studies with constitutive expression of erythropoietin (Kessler et al. 1996). Regulation over hours to days has been achieved by concomitant transfer of a gene encoding a transcription factor that responds to an oral drug and directs expression of the therapeutic gene (Bohl et al. 1997, Rendahl et al. 1998, Burcin et al. 1999, Ye et al. 1999). However, rapid regulation over minutes, as would be required for tight control of DM, has not been achieved.

The third step for systemic gene therapy for most cardiovascular disorders involves the ability to maintain the effect by the transfer into a long-lived cell or a stem cell. Stable expression for over 1 year has been achieved from muscle and liver, which have little turnover in adult animals. In contrast, expression has declined from fibroblasts that are implanted on a matrix.

• Thromboembolic Disease

Venous thrombosis occurs in 1 in 1000 people per year. The presence of a coagulation factor that cannot be rapidly inactivated, such as factor V-Leiden, or the heterozygous deficiency of an inhibitor of coagulation, such as PC, increases the risk of thrombosis in adults by 3- to 10-fold (Simioni et al. 1999, Zoller et al. 1999). Gene therapy for factor V-Leiden would require inactivation of the gene or mRNA that encodes the PC-resistant protein and is beyond our current capabilities. Gene therapy for heterozygous deficiencies would require very high levels of expression (50% of normal) and is unlikely to be instituted in the near future, as many patients will never experience a clot.

The homozygous deficiency of PC results in disseminated intravascular coagulation and frequent spontaneous thromboses in neonates. Levels of PC that are 5%–10% of normal can prevent these symptoms. Retroviral vector-mediated transfer of PC in rats recently was shown to result in >5% of normal PC levels for more than 1 year (Cai et al. 1998), which would be sufficient to prevent most spontaneous thromboses. Although this study used a partial hepatec-

tomy to facilitate gene transfer into the liver, hepatic growth factors are equally effective at promoting efficient gene transfer into the liver with no adverse effects. Because lifelong anticoagulation with coumadin or parenteral injection of PC is problematic, homozygous PC deficiency would clearly benefit from gene therapy.

An alternative approach to thromboembolic disease would be to use gene therapy to dissolve a clot once it developed. Adenoviral vector-mediated transfer of tissue plasminogen activator (t-PA) resulted in lysis of pulmonary emboli in mice that were deficient in t-PA or overexpressed plasminogen activator inhibitor 1 (PAI-1) (Carmeliet et al. 1997). However, it is unclear if this approach would be used in humans due to the potential risk of bleeding and the availability of recombinant protein for treatment of acute events.

• Hypercholesterolemia

Hypercholesterolemia is a multifactorial disorder that is a major risk factor for atherosclerosis, and lowering cholesterol levels can clearly decrease this risk (Pedersen 1998). Homozygous familial hypercholesterolemia (FH) is due to a deficiency of the low-density lipoprotein receptor (LDL-R) and results in cholesterol levels >600 mg/dL. Although adenoviral vector-mediated transfer of the LDL-R gene into the liver normalized cholesterol levels in mice (Ishibashi et al. 1993) and rabbits (Kozarsky et al. 1994, Li et al. 1995) with FH, this effect was transient. Retroviral vector-mediated gene transfer was marginally successful at lowering cholesterol levels in rabbits (Chowdhury et al. 1991) and humans (Grossman et al. 1995) with FH, presumably due to lower gene transfer efficiency or expression levels. Alternatively, adenoviral vector-mediated overexpression of proteins that promote cholesterol uptake into cells by alternative mechanisms, such as the very-low-density lipoprotein receptor (VLDL-R) (Kobayashi et al. 1996, Kozarsky et al. 1996a), lipoprotein lipase (Zsigmond et al. 1997), or the apo B mRNA editing enzyme (Kozarsky et al. 1996b) has lowered or normalized cholesterol levels in animals with FH for days to weeks [for a reviewed, see Gerard and Colten (1997)]. Similarly, adenoviral vector-mediated transfer of apo E (Kashyap et al. 1995, Stevenson et al. 1995, Tsukamoto

et al. 1997), apo AI (Benoit et al. 1999), and lipoprotein lipase (Zsigmond et al. 1997) transiently lowered cholesterol and inhibited atherosclerosis in apo-E-deficient mice. Long-term expression from vectors that are more stable has not been reported. Gene therapy for homozygous FH would be a major advance, as these patients respond poorly to pharmacologic treatment and die at a young age of atherosclerosis. It also might be applied to patients with hypercholesterolemia due to other causes. However, higher and more stable expression will need to be achieved before this approach will have a clinically significant effect in humans.

• Diabetes Mellitus

DM is a major risk factor for atherosclerosis, and intensive therapy appears to decrease the frequency of macrovascular events (Lawson et al. 1999). Type I DM is due to decreased insulin production because of immune-mediated destruction of the pancreatic β cell. Gene therapy with an insulin gene delivered via a retroviral vector to the liver (Kolodka et al. 1995) or from implanted fibroblasts (Taniguchi et al. 1997) improved fasting glucose levels in streptozocin-treated rats, but had little effect on glucose levels after feeding. Physiologic control of insulin production and release in response to blood glucose levels over minutes would need to be achieved for this approach to be implemented in humans.

• Hypertension

Hypertension is a common multifactorial disorder that is a major risk factor for atherosclerosis (O'Byrne and Caulfield 1998) and whose treatment can clearly decrease this risk (Hansson et al. 1998). Blood pressure was significantly decreased but was not normalized for a few weeks in hypertensive rats by the expression of atrial natriuretic peptide (Lin et al. 1998, 1999) or kallikrein (Chao et al. 1998, Yayama et al. 1998) from an adenoviral vector; and the risk of stroke, cardiac hypertrophy, and renal damage was reduced. Expression of antisense RNA for angiotensin II type 1 receptor after delivery to the heart of neonates via a retroviral vector (Martens et al. 1998) lowered blood pressure for more than 2 months. It is unclear, however, if gene therapy will play an important role in the treatment of

this disorder due to the availability of effective drugs and the potential risks of hypotension because of loss of physiologic control mechanisms.

• Summary

Systemic gene therapy might be used to prevent or treat cardiovascular diseases, such as thromboembolic disease, hypercholesterolemia, hypertension, or DM. Current gene therapy approaches with retroviral and AAV vectors are limited by their inability to achieve sufficient levels of expression for many disorders, whereas adenoviral vectors are limited by short-term expression. For the treatment of DM or hypertension, the ability to achieve physiologic regulation of expression will be necessary prior to using these treatments in humans. If these problems can be solved, systemic gene therapy may have a significant impact on cardiovascular diseases in the near future.

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Enhancement of Vitamin-K-Dependent Protein Function by Modification of the γ -Carboxyglutamic Acid Domain: Studies of Protein C and Factor VII

Gary L. Nelsestuen*

Vitamin-K-dependent proteins are found in both the pro- and anti-coagulation cascades, and their use in coagulation therapies is expanding rapidly. The vitamin-K-dependent, γ -carboxyglutamic acid (Gla)-containing regions of proteins in this family are homologous and are responsible for membrane association. Site-directed mutations that enhance the membrane affinity of protein C, an anticoagulant, and of factor VII, a procoagulant, have been identified. These protein C and Factor VII mutants show enhanced activity in many assays, offering opportunities to study the role of membrane in blood clotting reactions and proteins that may have greater therapeutic value. (Trends Cardiovasc Med 1999;9:162-167). © 1999, Elsevier Science Inc.

Recombinant vitamin-K-dependent proteins are finding increased use in therapy for blood coagulation disorders. Although the most obvious target is factor IX, the protein deficient in hemophilia B (Sadler and Davie 1987), others are the focus of emerging therapies. For example, high-dose factor VIIa is efficacious in treatment

of bleeding episodes in both hemophilia A and B (Hedner 1996, Hedner et al. 1993). Its major use is for patients with inhibitors of IX or VIII. Protein C, of the anticoagulant cascade (Esmon 1989), is a candidate for treatment of certain thrombotic states (Rintala et al. 1998). In addition, active site blocked VIIa (VIIai) is superior to other anticoagulants in many circumstances (Harker et al. 1997). A leading problem for general use of these recombinant proteins is cost. Owing to the need for vitamin-K-dependent carboxylation of glutamic acid residues, only carboxylation-competent animal cell lines may be used for protein production. Overexpression is limited because the carboxylase enzyme seems easily overwhelmed. Thus, proteins with enhanced activity

Gary L. Nelsestuen is at the Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, St. Paul, Minnesota, USA.

* Address correspondence to: Dr. Gary L. Nelsestuen, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, 1479 Gortner Avenue, St. Paul, MN 55108, USA.

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