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Liver-directed Gene Therapy for Dyslipidemia and Diabetes

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Abstract

This article provides an update of liver-directed gene therapy for dyslipidemia, reviewing papers published since 2002 and summarizing progress in gene transfer vectors. Despite the availability of polypharmacy and other therapeutic interventions, the treatment of severe dyslipidemia remains a challenge and continues to be an important target for experimental gene therapy. Gene therapy strategies that focus on long-term therapeutic efficacy of different regimens are emerging from small animal experiments, and new therapeutic genes and/or new approaches have been developed. A novel strategy for gene therapy for diabetes was published recently. Gene therapy for dyslipidemia and diabetes is still in its infancy. Nonetheless, recent progress in this area is encouraging and bodes well for the future.

Introduction

Despite recent advances in primary and secondary prevention [1], cardiovascular disease remains the leading cause of morbidity and mortality in industrialized countries. Furthermore, an aging population and an increasing prevalence of overweight and obese individuals in this country are sure to exacerbate the statistics. The development of cholesterol-lowering drugs, including niacin, a choice of different statins and fibrates, bile acid sequestrants, and a recently discovered cholesterol absorption inhibitor, has greatly increased our options in primary and secondary prevention. Nonetheless, there are limitations in using the currently available polypharmacy in combating established hypercholesterolemia and dyslipidemia [2]. Physician education of the importance of reaching therapeutic goals is one problem. Furthermore, a substantial portion of patients do not respond adequately to or tolerate these drugs, and are, therefore, unable to meet the National Cholesterol Education Program (NCEP) guidelines [1]. The need for novel therapies for these patients prompted the exploration of somatic gene therapy as an alternative approach. There has been substantial progress in our understanding of vascular biology and the pathogenesis of atherosclerosis in the past few years. The purpose of this review is to summarize the progress made toward the development of liver-directed gene therapy for dyslipidemia since the last review in 2002 [3]. We first review progress made in vector biology and discuss some safety issues before embarking on an update on publications related to treatment efficacy using some established genes as well as novel genes for the treatment of dyslipidemia. As diabetes is a major risk factor for atherosclerosis, we have reviewed a recently developed novel strategy for the treatment of diabetes using a gene therapy approach.

Progress in Gene Delivery Vectors

Although much progress has been made in nonviral vectors [4], viral vectors remain the major vehicle for liver-directed gene delivery. Over the past few years, significant progress has been made in viral vector technology and in the understanding of the molecular mechanism of virus infection. Much further work needs to be done in improving vector efficiency, specificity, and safety before gene therapy can fulfill its promise as the next breakthrough in therapy [5•]. Recently, the appearance of leukemia in two patients who received retroviral gene therapy for

one form of X-linked severe combined immunodeficiency [6] led to much discussion on the use of integrating viral gene systems for gene therapy [7]. The potential of insertional mutagenesis in gene therapy is a recognized risk, but its actual occurrence in two patients in one clinical trial raises a lot of concern. Most target cells in the liver are quiescent or undergo cell division very infrequently. Insertion mutagenesis leading to a proliferative advantage and tumor formation must be an extremely rare event in liver-directed gene therapy unless the transgene codes for a growth promoting protein. Retroviral and lentiviral vectors are traditional integrating vectors that favor insertion in active genes [8]. A viral vector that is absolutely non-integrating may not exist. For example, adenovirus-mediated transgene expression is mostly episomal. However, it has been reported that helper-dependent adenovirus (HDAd) vectors have the ability to integrate the transgene at an extremely low frequency, which is, however, substantially higher than that of conventional first-generation adenoviruses [9,10].

Development of targeted integration or of stable episomal vector systems may further improve their safety profile [11]. In terms of safety, nonintegrating or minimally integrating vectors such as adeno-associated virus (AAV) or adenovirus may be the preferred gene transfer vehicles, although without chromosomal integration transgene expression is transient in nature. To date, AAVs and adenoviruses remain the most popular vectors for in vivo gene transfer into the liver.

Adeno-associated Virus

Eight AAV serotypes have been isolated to date. For transgenes that require high-level expression for therapeutic efficacy, the relatively poor transduction efficiency is a limitation to the use of AAV serotype 2 (AAV2), the most popular serotype, as a vehicle for hepatic gene transfer. A new family of AAV vectors, based on AAV8 isolated from nonhuman primates, has been reported to be 100-fold more efficient in hepatic transgene expression compared with AAV2 [12]. Because the efficiency of AAV8 hepatic transduction was not influenced by prior immunization with the other AAV serotypes, it is unlikely that humans have neutralizing antibodies against vectors based on AAV8, a fact that may prove advantageous in human gene therapy. A practical problem of AAV vectors is the relatively long latent period between vector delivery and peak transgene expression. This lag period in gene expression occurs because conventional recombinant AAV vectors deliver a single-stranded DNA genome, which must be converted to double-stranded DNA before the transgene is expressed. The problem has been solved in part by the development of a self-complementary AAV vector system. However, this modification further limits the cloning capacity of AAV vector because it utilizes the fact that AAV can package the genomes half the size of the wild-type genome into the capsids either as two copies or as dimeric inverted repeat DNA [13]. Despite some limitations, AAV is becoming a popular viral gene delivery system. It should be noted that long-term gene expression mediated by AAV was originally thought to be related to its ability to integrate into the host chromosome [14]; however, now it is generally believed that the vector persists long-term as an episome [15]. Even though the integration of AAV vector genome into the host genome is biased toward active genes [16], the tumorigenic potential resulting from insertional mutagenesis is minimized in liver-directed gene therapy for reasons given previously.

Helper-dependent Adenoviral Vectors

There is substantial ongoing effort directed at improving the safety profile of first- or second-generation adenovirus vectors and prolonging transgene expression following transduction. The toxicity and immunogenicity of these early-generation adenoviruses can be used to advantage for some applications, such as cancer gene therapy and vaccination. Although dyslipidemia can lead to devastating long-term consequences (as the disorder is not immediately life threatening), there is little justification for use of toxic early-generation adenoviruses for the treatment of dyslipidemia [3].

In liver-directed gene therapy, systemic vector administration is the ideal route for global hepatocyte transduction. Despite reduced host-adaptive immune responses against vectors and improved gene transfer efficiency in HDAd vectors, it is impossible to avoid innate responses shortly after vector administration in vivo. The innate immune response to HDAd vectors may underlie the acute toxicity of all adenoviruses [17], resulting from the direct interaction of viral particles with innate immune effector cells, Kupffer cells, and other cells of the reticuloendothelial system [18]. Schiedner *et al.* [19] found that transient depletion of Kupffer cells before vector injection increased hepatic transduction efficiency of HDAd vectors. Another approach to reduce toxicity and increase liver-specific gene delivery may be by local vector administration. A catheter-based gene delivery system has been developed for direct delivery of plasmid DNA to the liver of rabbits [20]. Despite a greatly improved safety profile and long-term transgene expression, HDAd vectors are difficult to step up, as the large-scale production of these vectors remains a practical problem. Two studies have addressed this problem using suspension cell culture systems [21,22].

Dyslipidemia and Therapeutic Genes

A number of potential therapeutic genes have been proposed and tested for their efficacy in experimental gene therapy for animal models of dyslipidemia. Recent data suggest that it is important to examine the effect of long-term transgene expression, as results from such experiments seem to differ significantly from those obtained from short-term transient transgene expression experiments [23•]. This is to be expected as atherosclerosis is a slowly progressive disease that usually takes decades to develop, and trials using any potential therapeutic agent must be monitored for extended periods of time.

In addition to the dyslipidemia that bathes the atherosclerotic lesions, local inflammation in the arterial wall is now recognized to be an important pathogenic factor in atherosclerosis development [24]. Therapeutic genes that affect atherosclerosis associated with dyslipidemia can be classified into two broad categories: 1) those that lower atherogenic lipoproteins, elevate antiatherogenic lipoproteins, or facilitate reverse cholesterol transport; and 2) genes that modulate the inflammatory process (Table 1).

Therapeutic genes that decrease atherogenic lipoproteins

LDL receptor and VLDL receptor—Familial hypercholesterolemia (FH) is a common inherited disorder caused by defective low-density lipoprotein receptor (LDLR). Despite a variety of primary and secondary interventions, a substantial number of homozygous LDLR-deficient patients fail to respond to these treatments [25]. Therefore, FH is a good target disease for gene therapy. The efficacy of long-term LDLR gene therapy to treat hypercholesterolemia in LDLR^{-/-} mice has been reported in abstract form. Leberherz *et al.* [26] produced vectors based on AAV2, AAV2/7 (AAV vector made of capsids from AAV7 using rep and inverted terminal repeats from AAV2), and AAV2/8 expressing LDLR driven by a liver-specific promoter. LDLR^{-/-} mice fed a Western-type diet were then treated with these vectors [26]. Serum cholesterol levels were significantly reduced and the effects were sustained for 20 weeks, though the AAV2-based vector was less effective. This is in contrast to the earlier study using AAV2 with a ubiquitous promoter [27] in which LDLR expression resulted in a transient modest reduction of cholesterol due, in part, to the generation of anti-LDLR antibodies. These results confirm that the use of a liver-specific promoter may evade the humoral immune response against newly synthesized LDLR. Mice treated with AAV2/7 had 68% fewer atherosclerotic lesions than control mice, whereas those treated with AAV2/8 had 56% fewer lesions. This result was not expected, as AAV2/8 had been reported to be 10-fold more effective in liver expression than AAV2/7-based vector [12].

Another study used HDAd vector expressing monkey LDLR driven by a liver-specific promoter and compared its efficacy with that of very low-density lipoprotein receptor (VLDLR), which is a surrogate receptor that is normally expressed in tissues other than liver and thus circumvents the host immune response. LDLR^{-/-} mice were fed a high-cholesterol diet for 20 weeks before virus treatment to induce atherosclerosis. Although 20% of LDLR^{-/-} mice treated with a high dose of HDAd-LDLR developed anti-LDLR antibodies, LDLR gene therapy was more effective than VLDLR gene therapy both in cholesterol lowering and in inhibiting the progression of atherosclerosis. Despite the cholesterol lowering for 24 weeks in mice treated with HDAd vectors, the induction of regression of the advanced lesions was not observed in any treatment groups. The hypocholesterolemic effects of HDAd-LDLR treatment lasted over 1 year, and at 71 weeks the cholesterol levels were 260 mg/dL in treated mice versus 488 mg/dL in control mice ($P < 0.01$) [28].

Lipoprotein lipase—Genetic deficiency of lipoprotein lipase (LPL) (type 1 hyperlipidemia) causes chylomicronemia associated with low high-density lipoprotein (HDL) cholesterol because LPL plays a pivotal role in hydrolysis of triglyceride-rich lipoproteins, such as VLDL and chylomicrons, and provides surface components for HDL formation. Although LPL is mainly synthesized in adipose tissue, heart, and muscle, adenovirus-mediated ectopic hepatic expression has been reported to correct the phenotype in a feline model of LPL deficiency [29]. The development of gene therapy for genetic LPL deficiency using AAV has been reported in abstract form. The intramuscular (IM) injection of pseudotype AAV1 expressing natural variant LPLS447X, a common LPL variant associated with a beneficial lipid profile, corrected hypertriglyceridemia in LPL^{-/-} mice for a year [30]. The same vector used to infect skeletal muscle isolated from patients with LPL deficiency was effective in inducing LPL expression, suggesting that the approach may be feasible in humans with LPL deficiency [31]. Hepatic LPL overexpression produced by first-generation adenovirus-mediated gene transfer had been shown to be effective in reducing plasma cholesterol and triglyceride in apolipoprotein (apoE) ^{-/-} and LDLR^{-/-} mice [32], but its use in later-generation vectors for this purpose has not been reported.

Therapeutic genes that increase antiatherogenic lipoproteins

Apolipoprotein A-I—Early studies using a first- or second-generation adenovirus have shown that short-term hepatic apoA-I expression (lasting a few weeks) was sufficient to induce fatty streak lesion regression. HDAd-mediated expression of apoA-I for 24 weeks at levels similar to human plasma inhibited the progression of established lesions in LDLR^{-/-} mice, but did not induce true lesion regression. Instead, the treatment remodeled lesions that contained abundant macrophages, vascular cell adhesion molecule-1 (VCAM-1), and a thin fibrous cap to a more stable-appearing phenotype [23•]. These results suggest that apoA-I gene therapy is an effective way to reduce acute coronary syndromes, which are often caused by rupture of vulnerable plaques [33,34].

ATP-binding cassette transporter A1—ATP-binding cassette transporter A1 (ABCA1) is critical in apoA-I-mediated cholesterol and phospholipid efflux. It plays an important role in HDL maturation and reverse cholesterol transport by mediating lipid efflux from macrophages. Genetic deficiency of ABCA1 is the basic defect of Tangier disease, a low HDL syndrome associated with accelerated atherosclerosis [35,36]. Increased ABCA1 activity has been reported to protect against atherosclerosis in mice [37,38]. Although macrophage-specific apoE expression plays an important role in this protection [37], apoA-I expression in macrophages can compensate for an absence of apoE [39]. Adenovirus-mediated hepatic ABCA1 expression in C57BL/6 wild-type mice led to significant increase in apoA-I-dependent cholesterol efflux, accompanied by increase in plasma concentrations of total cholesterol, phospholipids, free cholesterol, HDL cholesterol, apoE, and apoA-I. The treatment also

increased the expression of genes for 3-hydroxy-3-methylglutaryl coenzyme A reductase, LDLR, and LDLR-related protein [40,41]. These results underscore the importance of regulated ABCA1 expression and carefully selected target tissues when ABCA1 is used for gene therapy.

Lecithin:cholesterol acyltransferase—Lecithin:cholesterol acyltransferase (LCAT) mediates the esterification of free cholesterol on plasma lipoproteins. This step converts discoidal, nascent HDL particles into mature spherical HDL. Obesity-associated dyslipidemia in humans is often associated with insulin resistance and accelerated atherosclerosis. Mertens *et al.* [42•] developed leptin-deficient (ob/ob) LDLR^{-/-} mice in which the oxidative stress was increased and HDL-associated antioxidant defense was impaired. They treated these mice with early-generation adenovirus expressing LCAT. The “atherosclerosis” (measured as plaque volume in the aortic root) was reduced by 42% at 6 weeks after the treatment.

Endothelial lipase—Endothelial lipase (EL) is a recently identified member of the vascular lipase gene family, which also encompasses LPL and hepatic lipase (HL) and plays a major role in HDL metabolism [43–45]. Although both HL and EL have phospholipase activity, HL may act primarily on the triglyceride-rich HDL particle, whereas EL may act primarily on HDL phospholipids [46]. Hepatic overexpression of EL in mice resulted in markedly reduced HDL cholesterol and apoA-I levels, which was associated with an increase in postheparin plasma phospholipase activity, HDL-apolipoprotein catabolism, and apoA-I uptake in kidney and liver [43,46]. Adenovirus-mediated overexpression of catalytically inactive EL led to a modest reduction of total plasma cholesterol, HDL cholesterol, and phospholipids in HL-deficient mice, suggesting that EL may possess both a lipolytic and nonlipolytic function in HDL metabolism [47]. Reduction of the maturation of nascent HDL could inhibit reverse cholesterol transport, whereas an increase of HDL catabolism may accelerate cholesterol disposal. Therefore, hepatic overexpression of EL may have both proatherogenic and antiatherogenic consequences. Additional experiments are necessary to clarify its overall long-term effect on atherosclerosis.

Combination therapy: the more, the better?—A reduction in atherogenic lipoproteins and an increase in antiatherogenic lipoproteins protect against atherosclerosis through overlapping mechanisms [24]. A reduction in LDL cholesterol or an increase in HDL cholesterol can independently reduce the risk of cardiovascular disease. There is considerable interest in the outcome of combined therapy and whether therapies that take advantage of different pathways might have synergistic or additive effects on atherosclerosis. ApoE functions as an atherogenic as well as an antiatherogenic protein. Although hepatic expression of human apoE3 mediated by a second-generation adenovirus alone inhibited atherosclerosis progression in LDLR^{-/-} mice, the addition of LDLR to the apoE3 regimen failed to show an additive or synergistic effect on cholesterol lowering and atherosclerosis [48••]. In contrast, apoA-I’s antiatherogenic effect is well established. Using first- and second-generation adenovirus vectors, Kawashiri *et al.* [49] tested the combined effects of LDL cholesterol reduction by LDLR expression and HDL cholesterol enhancement by apoA-I expression in LDLR^{-/-} mice fed a Western-type diet. Transgene expression was transient and the effects of virus treatment were detected for only 2 weeks. The atherosclerotic lesion areas (mainly of fatty streaks) were reduced by 45% in LDLR-treated mice, 42% in apoA-I-treated mice, and 37% in mice treated with both vectors. In all treatment groups, the areas stained for macrophages were reduced to a similar extent. One plausible explanation for this result is that LDL cholesterol lowering and increasing apoA-I influence atherosclerosis through either similar mechanisms or partially overlapping mechanisms. The inconclusive nature of such short-term experiments indicates the importance of long-term experiments before the potential utility of combined therapy can be interpreted with confidence.

Class A scavenger receptor—Macrophage scavenger receptor (SR) type AI is a member of the SR family that consists of integral membrane glycoproteins. Class A SR (SR-A) mediates the uptake of modified lipoproteins and plays an important role in atherosclerosis development. Jalkanen *et al.* [50] produced AAV expressing a secreted form of macrophage SR type AI, which functions as a decoy and inhibits the uptake and degradation of modified LDL by macrophages, thereby inhibiting foam cell formation. The authors injected the vector into LDLR^{-/-} mice via the tail vein and found a modest 21% reduction of atherosclerotic lesion area after 6 months; however, no difference in lesion composition was detected [50].

Genes that modulate the inflammatory process

Adiponectin—Adiponectin is an adipocytokine whose plasma concentration is inversely correlated with plasma triglycerides and positively correlated with HDL cholesterol level. Obesity and obesity-related abnormalities, such as insulin resistance, dyslipidemia, and type 2 diabetes, are associated with a chronic inflammatory state. Adiponectin levels are also negatively correlated with inflammatory markers such as C-reactive protein. Potential antiatherogenic actions of adiponectin include inhibition of tumor necrosis factor α (TNF- α)—induced expression of endothelial adhesion molecules, smooth muscle cell proliferation and migration, and TNF- α -mediated signaling pathway [51]. Okamoto *et al.* [52•] treated apoE^{-/-} mice with a first-generation adenovirus expressing adiponectin. After 14 days, the lesion formation in the aortic sinus of mice treated with the vector was 30% smaller than that treated with the control virus. Interestingly, adiponectin co-localized with macrophages in the fatty streak and significantly suppressed VCAM-1 and SR-A expression [52•].

Gene Therapy of Diabetes

Diabetes is one of the most important risk factors for atherosclerosis. The atherogenic effect of the diabetic state appears to be independent of changes in plasma lipoproteins. Despite the recent development of new forms of insulin with widely varying durations of action and the availability of insulin-sensitizing agents that greatly improve insulin resistance, the treatment of diabetes remains unsatisfactory. Somatic gene therapy is one experimental approach that offers the promise of a “cure.” A recent review on this topic should be consulted for details [53•]. We briefly summarize here a recent development that may represent a conceptual breakthrough in therapy. Kojima *et al.* [54••] used HDAd to deliver a combination of a pancreatic islet-specific transcription factor (beta2/NeuroD) and an islet growth factor (betacellulin) to the liver of mice with streptozotocin-induced diabetes. They found that the treatment reversed diabetes in these mice. Treated animals became normoglycemic and started to gain weight. Further analysis showed that the mice had developed clusters of newly formed pancreatic islets in the liver that produced insulin as well as other islet hormones. Ultrastructural analysis revealed the presence of endocrine-like cells in the liver that contained insulin granules inside secretory vesicles. The fact that the mice exhibited a normal glucose tolerance test suggests that the insulin secretion by the ectopic islets was under the control of regulated exocytosis. The diabetes reversal appeared to be stable and lasted at least 4 months, which was the duration of the experiment. Further refinement of the experimental approach could revolutionize the treatment of insulin-deficient diabetes.

Conclusions

Gene therapy was conceived with great expectations as the latest frontier in tissue-specific and disease-targeted therapy. Since its inception, its development has had its fits and disappointments. In the areas of dyslipidemia and diabetes, liver-specific gene delivery has enabled us to test different regimens and design some important proof-of-concept experiments. In the past year, data on the long-term effect of gene therapy in small animal models have become available, an important step in the development of novel treatment for these chronic

disorders. Although the results are encouraging, much additional work remains to be done to further improve the safety profile of gene delivery vectors, optimize routes of administration, and refine regulated transgene expression. We are optimistic that we can move these treatments from the bench to the bedside in the near future.

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Table 1

Therapeutic genes for gene therapy of dyslipidemia and their effects on lipoprotein metabolism and atherosclerosis

Gene	Effect	Study
Genes that reduce atherogenic lipoproteins		
LDLR	AAV2/7 and AAV2/8 expressing human LDLR inhibited atherosclerosis in LDLR ^{-/-} mice fed a Western-type diet, but AAV2/7 was more effective than AAV2/8 vector	Lebherz <i>et al.</i> [26]
VLDLR	HDAd expressing LDLR was more effective in cholesterol lowering and inhibiting atherosclerosis progression than HDAd expressing VLDLR in LDLR ^{-/-} mice	Oka <i>et al.</i> [28]
LPL	Intramuscular injection of AAV1 expressing natural variant LPLS447X corrected hypertriglyceridemia in LPL ^{-/-} mice for 12 months. This approach has been tested for its feasibility in patients.	Ross <i>et al.</i> [30] and Rip <i>et al.</i> [31]
Genes that increase antiatherogenic lipoproteins		
ApoA-I	The expression of human apoA-I using HDAd vector for 24 weeks inhibited atherosclerosis lesion progression in LDLR ^{-/-} mice and remodeled the unstable looking lesions to a more stable phenotype	Belalcazar <i>et al.</i> [23••]
ABCA1	Hepatic overexpression of ABCA1 led to complex effects on lipoprotein metabolism, which underscores the importance of regulated ABCA1 expression in appropriate target tissues	Basso <i>et al.</i> [40] and Wellington <i>et al.</i> [41]
LCAT	Overexpression of LCAT in the liver of leptin-deficient LDLR ^{-/-} mice resulted in the reduction of atherosclerosis in the aortic root area	Mertens <i>et al.</i> [42•]
EL	Hepatic overexpression of catalytically inactive EL revealed its nonlipolytic function in HDL metabolism	Broedl <i>et al.</i> [47]
Combination LDLR / ApoA-I	Cholesterol-fed LDLR ^{-/-} mice were treated with adenovirus expressing LDLR or apoA-I and their effects on atherosclerosis were studied. No additive or synergistic effects were observed in mice treated with both vectors.	Kawashiri <i>et al.</i> [49]
Novel approach SR-A	Secreted class A SR using AAV vector was used as a decoy to compete with modified LDL. The modest 21% reduction of the lesion area was reported.	Jalkanen <i>et al.</i> [50]
Genes that work through inflammatory pathway		
Adiponectin	Adenovirus-mediated hepatic expression of adiponectin inhibited atherosclerosis progression in apoE ^{-/-} mice	Okamoto <i>et al.</i> [52•]

AAV—adeno-associated virus; ABCA1—ATP binding cassette transporter A1; apo—apolipoprotein; EL—endothelial lipase; HDAd—helper-dependent adenovirus; HDL—high-density lipoprotein; LCAT—lecithin:cholesterol acyltransferase; LDLR—low-density lipoprotein receptor; LPL—lipoprotein lipase; SR-A—class A scavenger receptor; VLDLR—very low-density lipoprotein receptor.