Alipogene tiparvovec: gene therapy for lipoprotein lipase deficiency

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Homozygous lipoprotein lipase (LPL) deficiency is an ultra-orphan disease associated with increased rates of pancreatitis. Current treatments based on acute plasmapheresis allied with ultra-low fat diets are inadequate as responses to fibrates or other triglyceride-lowering therapies tend to be poor. Alipogene tiparvovec is an adeno-associated virus type I (AAV1) gene therapy using a hyper-functional LPL serine447-stop (S447X) insert administered intramuscularly under general anaesthetic with allied immunosuppression. Treatment results in histological muscle expression of LPL allied with a transient 40% reduction in triglycerides and improvements in postprandial chylomicron triglyceride content. Alipogene tiparvovec is the first possibly curative treatment for LPL deficiency.

Keywords: alipogene tiparvovec, chylomicronemia, gene therapy, lipoprotein lipase, triglyceride

1. Introduction

Severe hypertriglyceridaemia is the only emergency in lipidology [1,2]. It is associated with a risk of pancreatitis that increases markedly with plasma triglyceride (TG) concentrations above 20 mmol/L though most cases occur with TG > 35 mmol/L [3]. Its aetiology is complex [1] but includes a functional or genetic deficiency in lipoprotein lipase (LPL) allied with secondary factors that either increase TG production, for example, alcohol or impair the clearance of TG-rich lipoproteins, for example, insulin resistance or diabetes. Lipoprotein lipase is a liver and muscle-secreted enzyme present on the vascular endothelial surface that hydrolyses surface TG from particles that show low relative amounts of phospholipid in contrast with endothelial or hepatic lipases. The function of LPL as an enzyme is modulated by apolipoprotein (apo) C2 as a cofactor, and by apoA5 and lipid maturation factor-1 (LMF-1) as a regulator of hepatic expression [4]. In addition, LPL transfer to the surface of TG-rich particles and a receptor—glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPI-HBP1) is involved in clearance of these opsonised particles. Thus, deficiencies in any one of five proteins can cause functional LPL deficiency.

Severe hypertriglyceridaemia associated with LPL deficiency occurs in 1 in 20,000 to 1 in 1,000,000 depending on the exact nature of the defect. Heterozygotes for LPL deficiency (actually 1% of the population) requires secondary factors such as age, smoking, insulin resistance or excess alcohol intake to cause a hypertriglyceridaemic state [5]. The TG-rich particles in these cases consist mostly of very low density lipoprotein (VLDL) with some chylomicron (CM) remnants and are associated with a type IV or V hyperlipidaemia. In contrast in homozygous LPL deficiency, CM metabolism is impaired and very little VLDL is produced by the liver, thus giving rise to a type I hyperlipidaemia.
2. Lipoprotein lipase deficiency

Most cases of LPL deficiency have a type IV – type V phenotype and are caused by the interaction of heterozygosity for LPL deficiency with environmental factors [5]. These patients typically present at age 50 with either eruptive xanthomata or pancreatitis. They often have insulin resistance or type 2 diabetes. A bout of acute pancreatitis event is often triggered by a respiratory tract or other infection. Their cardiovascular risk is variable but often increased but the principal risk of type 2 diabetes secondary to beta cell destruction secondary to episodes of acute and sub-acute pancreatitis. The development of diabetes exacerbates any underlying TG elevation further. Most patients with this phenotype respond to a combination of fibrate; omega-3 fatty acid, niacin and statin therapy allied with hypoglycaemiac drugs [1].

By contrast, patients with the type I phenotype present in childhood with hypertriglyceridaemia [6] allied with abdominal pain and pancreatitis even with diets relatively low in saturated fat and TGs. Unlike patients with type IV/V, they often show relative adipose tissue depletion with no evidence of central obesity. Their cardiovascular disease risk is low but they have a high probability of developing secondary type 2 diabetes. They pose a therapeutic challenge as their response to fibrates or other TG-lowering drugs is decreased or even absent. The mainstay of treatment in type I is a diet ultra-low in saturated fat (< 10 g/day) but they also show reduced CM production after treatment with orlistat to reduce gastric lipase activity and intestinal fatty acid uptake.

The very large CM particles present in type I and type V hyperlipidaemia cause a hyperviscosity syndrome and patients can complain of vasculitis and transient ischaemic attacks. Rates of subarachnoid haemorrhage are also increased in patients with severe hypertriglyceridaemia.

3. Therapeutics of LPL deficiency

The primary therapy for moderate (heterozygous) LPL deficiency are fibrate drugs that activate peroxisomal proliferator activating receptor alpha (PPAR-α). This nuclear receptor activates synthesis of LPL, often apo A-1 but also reduces the synthesis of apoC3 which acts as a negative regulator of LPL activity. Similarly, some thiazolidinedione drugs (i.e., pioglitazone) have some PPAR-α as well as insulin-sensitising PPAR-γ actions and can reduce TGs. Omega-3 fatty acids act through a specific GPR120 receptor to reduce inflammation and PPAR-γ to insulin sensitise but also have other unclear effects that lead to reduced VLDL synthesis [7]. Niacin reduces free fatty acid flux to the liver via GPR109 but mostly acts through diacylglycerol acyltransferase (DGAT)-2 to reduce lipidation of CM and VLDL precursors [8]. Statins reduce TGs by upregulating the LDL receptor whose principal actions are to enhance clearance of apoB-100 containing lipoproteins but also to clear particles containing apoE, that is, TG-rich particles.

In acute management, cessation of food intake is also used to stop the production of postprandially secreted lipoproteins from the gut. In very severe acute cases, excess TG-rich lipoproteins can be removed physically by plasmapheresis or haemodialysis.

In cases of severe LPL deficiency (sometimes homozygous) dietary therapy with extreme limitation of saturated fat intake is the mainstay of treatment. Fibrates, omega-3 fatty acids and niacin may have some effect but this is often reduced when compared with the effects seen in heterozygotes. As dietary fat is the source of the lipid incorporated into the CMs present in LPL deficiency, other approaches that interfere with either gut absorption of fatty acids, or with CM synthesis may offer benefits in LPL deficiency. Orlistat, a gastric lipase inhibitor which reduces fat availability, has been used successfully in the treatment of moderate and severe LPL deficiency [9,10].

4. Therapeutic applications of the enzyme LPL

Numerous single nucleotide polymorphisms (SNP) have been identified in LPL and some have been associated with increased TG concentrations and an increased risk of CVD in epidemiological studies. However, one SNP—S447X, present in 20% of Caucasians, was found to be associated with increased activity of the LPL enzyme caused by increased expression of LPL mRNA and reduced sensitivity to inhibition by apoC3 [11]. This SNP was also associated with an improved response to low fat diets and to fibrate therapy. This LPL variant has been used as the basis of LPL gene therapies given its proven stability, efficacy and increased activity compared to the normal form.

To investigate gene therapy, animal models are required which mimic the human phenotype of disease accurately. Animal models of LPL deficiency exist including specific gene knockout mice but also on a larger-scale LPL-deficient Burmese cats. All these animal models suffer from severe hypertriglyceridaemia associated with acute and chronic pancreatitis. Potential methods to rescue the phenotype in these animals and in man could include infusion of the deficient enzyme—analogous to treatments used for lysosomal storage disorders or Pompe’s disease but the half-life of LPL is 15 min in plasma. Alternatively, a novel source of active LPL could be provided. Liver transplantation for LPL deficiency using cell infusion-based systems or lobe transplant remain a possibility for treatment of LPL deficiency analogous to the approach used in homozygous-familial hypercholesterolaemia. However, these would only restore LPL activity to the liver and peripheral vasculature and there is also abundant LPL in muscle where it may have a role in muscle-based lipid metabolism.

5. Alipogene tiparvovec

There have been previous attempts at gene therapy for inherited metabolic diseases. These have failed either due to
toxicity—adenovirus-induced syncytial pneumonitis in trial in cystic fibrosis, hepatic toxicity—ornithine transcarbamylase deficiency or more frequently failed due to inadequate efficacy with decline of expression of the constructs after 1–2 months, for example, in homozgyous familial hypercholesterolaemia. Novel viral vectors based on adeno-associated virus (AAV) have been engineered to give different tissue specificities and enhanced stability of expression [12].

Alipogene tiparvovec (AAV-LPL; Amsterdam Medical Therapeutics (AMT), then uniQure) is an AAV1-based gene therapy containing the LPL S447X gene construct with a constitutive expression promoter. In animal models of both mice [13] and cats [14] this therapy restored histologically detectable LPL activity, reduced TGs and improved lipoprotein turnover with few obvious side effects. Studies have recently been conducted in man in eight patients with homozygous LPL deficiency from the Netherlands using two doses [15] and then at higher dose in 14 patients with homozygous LPL deficiency from the Netherlands and Quebec [16,17]. Patients with LPL deficiency in Quebec show a restricted mutation spectrum due to founder effects [4]. Only patients with genetic LPL deficiency were recruited for studies with AAV-LPL. Presence of apoC2, apoA5 or GPI-HBP1 mutations were specific exclusion criteria for the trials. The AAV-LPL trials were conducted at two different doses (3 x 10^{11} or later mostly with 1 x 10^{12} genome copies/kg) with/without a 12-week course of immunosuppressants started prior to injection [17]. The therapy was given as 64 intra-muscular 1 ml injections in the major skeletal muscle groups under ultrasound guidance and performed with pain relief and under a general anaesthetic as a day case procedure. Lipid profiles, lipoprotein turnover and muscle biopsies were performed to monitor progress over up to 1 year. The lowest dose of AAV-LPL reduced TGs transiently by 20–30% and showed poor muscle expression. Subsequent studies using a higher dose, gave demonstrable maintained injection site histological expression. Immunosuppression was added to the pretreatment protocol to reduce T-cell-mediated anti-viral responses [18]. These modifications resulted in an up to 40% reduction in the primary end point of plasma TGs at 3–6 weeks from initial levels of 25–40 mmol/L. However, this effect waned and TGs returned to previous values after 12 weeks. More detailed lipoprotein analysis showed a reduction in labelled CM production and CM TG content with AAV-LPL but no obvious effect on CM clearance or VLDL production or clearance [19]. Insulin sensitivity, non-esterified fatty acids; glycerol levels and safety markers (hepatic enzymes, creatine kinase) were unchanged. A comparison of historic with post-treatment rates of pancreatitis admission suggested a reduction in the number and possibly the severity of pancreatitis events. These data for an ultra-orphan disorder with no other efficacious treatment were sufficient for AAV-LPL to be licensed by the European Medicines Evaluation Agency after an initial negative expert review.

6. Expert opinion

Homozgyous LPL deficiency is an ultra-orphan disorder with no good modern treatment. It results in considerable burden in morbidity due to pancreatitis and increased mortality related to both pancreatitis and hyperviscosity syndromes caused by CM plugs in capillary beds. Current dietary treatment is inadequate, drug response is poor and plasmapheresis only delivers transient benefits. Therapy with AAV-LPL does reduce TGs transiently in patients with genetically proven LPL gene deficiency and also seem to improve CM metabolism but only in demonstrating reduced production rate of new CMs. The data do not show an increase in CM clearance rates as might be expected for a highly efficacious therapy for LPL deficiency. Clinically, the data to date show lower rates of pancreatitis based on a comparison of pre- and post-treatment admission rates in a small number of subjects. The AAV-LPL treatment is suggested to reduce rates of pancreatitis admission by 60–70% compared with prior rates, but this type of cohort data can be confounded by other factors such as improved dietary adherence (or reduction in alcohol intake) under the close clinical supervision seen in clinical trial settings. The AAV-LPL treatment protocol is highly invasive and requires monitoring of the allied immunosuppressant therapy. The use of a gene therapy requires special site licensing for storage and administration of the vector and a specialist registry with good follow-up is essential to monitor the long-term efficacy and safety of this intervention. The only patients’ studies so far have come from a limited mutation spectrum in the LPL gene—mostly the four mutations found in Quebec and the response of other LPL mutations to this treatment is unknown. Though > 80% of LPL deficiency is caused by mutations in the LPL gene, the utility of AAV-LPL therapy has not been investigated in apoA5 deficiency where hepatic regulation of secretion seems to be the primary defect and in the milder phenotype of ApoC2 deficiency—where the lack of a cofactor for the active enzyme already present means it may not work. Similarly, addition of extra LPL in patients lacking the LPL receptor—GPI-HBP1 treatment with AAV-LPL—is not likely to lead to benefit but again no studies have been performed in animal models or later man for this indication.

Alternative approaches not relying on gene therapy also exist for the potential treatment of LPL deficiency. The most promising are based on agents that reduce TG-rich particle synthesis. These agents though primarily directed at hepatic VLDL production also can reduce CM synthesis in enterocytes and acute postprandial hypertriglyceridaemia. Microsomal transfer protein inhibitors (MTPIs) and especially those specifically directed to the gut (e.g., SurfaceLogix Sx4090) reduce CM production and may have a role in the treatment of LPL deficiency [20]. However, no studies of MTPIs in LPL-deficient patients exist as yet. Another approach that may have potential in reducing CM synthesis is the inhibition of diacylglycerol-acyl-transferase-1 (DGAT-1) but again no human studies have been performed as yet [21].
The proposed therapy using AAV-LPL is expensive with costs likely to be in the range of €250,000 ($300,000) payable for each of 5 years with additional costs for specialist clinics and investigations. In health systems that use health economic models to determine willingness to pay thresholds, the application for AAV-LPL will be controversial. Health economic models often have difficulties caused by wide confidence intervals due to lifetime extrapolation errors for conditions treated in adolescence/young adulthood. Current health economic cost effectiveness models also tend to use different discount rates for treatments likely to be only disease modifiers (typically 6%) from those that are potentially curative (3%) and it is uncertain whether AAV-LPL is a completely curative treatment. Long-term follow-up data will be essential to determine into which category AAV-LPL therapy fits and thus as to whether it is a cost-effective intervention even using the relaxed quality adjusted life year (QALY) criteria often applied to inherited errors of metabolism and orphan diseases.

**Declaration of interest**

AS Wierzbicki served on the advisory board for Amsterdam Medical Therapeutics (now uniQURE BV) from 2008 to 2009. The authors have no other competing interests to declare and have received no funding in preparation of the manuscript.

**Bibliography**


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