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Review

Genetic therapies to lower cholesterol

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ABSTRACT

This review surveys the state-of-the-art in genetic therapies for familial hypercholesterolaemia (FH), caused most commonly by mutations in the LDL receptor (*LDLR*) gene. FH manifests as highly elevated low density lipoprotein (LDL) cholesterol levels and consequently accelerated atherosclerosis. Modern pharmacological therapies for FH are insufficiently efficacious to prevent premature cardiovascular disease, can cause significant adverse effects and can be expensive. Genetic therapies for FH have been mooted since the mid 1990s but gene replacement strategies using viral vectors have so far been unsuccessful. Other strategies involve knocking down the expression of Apolipoprotein B100 (APOB100) and the protease PCSK9 which designates LDLR for degradation. The antisense oligonucleotide mipomersen, which knocks down APOB100, is currently marketed (with restrictions) in the USA, but is not approved in Europe due to its adverse effects. To address this problem, we have devised a novel therapeutic concept, APO-skip, which is based on modulation of APOB splicing, and which has the potential to deliver a cost-effective, efficacious and safe therapy for FH.

Contents

Familial hypercholesterolaemia (FH) is a common disease, characterised by low density lipoprotein (LDL) cholesterol and premature atherosclerosis. Normally, the liver assembles very low density lipoprotein (VLDL) particles from the full-length isoform of Apolipoprotein B (APOB100) plus triglycerides, cholesterol and cholesteryl esters in the rough endoplasmic reticulum and the Golgi. After secretion, the VLDL particles are metabolised by peripheral tissue lipoprotein lipase into intermediate density and LDL particles (Fig. 1A). LDL particles are then cleared via the LDL receptor (LDLR) in the liver.

FH is caused by mutations in *LDLR*, gain of function mutations in the serine protease PCSK9 which binds and designates LDLR for lysosomal degradation or accessory proteins such as LDLRAP1, which lead to loss-of-function of the LDLR. As a result, the normal clearance of LDL particles by binding of Apolipoprotein B100 (APOB100) to the LDLR on the cell surface of hepatocytes is disrupted, leading to accumulation of LDL particles in circulation which drive accelerated atherosclerosis (Fig. 1B). A similar physiological and clinical picture is observed in patients with familial defective *APOB* (FDB) who possess point mutations in the *APOB* gene that disrupt binding of APOB100 to the LDLR [1].

Heterozygous FH (HeFH) is the most common form, in which patients inherit one mutant allele of a causative gene, and is characterised by elevations of LDL cholesterol >4.9 mmol/L [2]. Homozygous FH (HoFH) is the most severe form in which patients inherit two mutant alleles, usually of *LDLR*. Similar severe HoFH clinical pictures are also seen when patients inherit two different mutant alleles of the same gene (compound heterozygosity) or two mutant alleles impacting different causative genes (e.g., *LDLR* plus one of either *PCSK9*, *LDLRAP1* or *APOB* — double heterozygotes). HoFH is characterised by even higher LDL cholesterol levels >13 mmol/L, and a first presentation of myocardial infarction typically in adolescence [3,4]. Although the prevalences of

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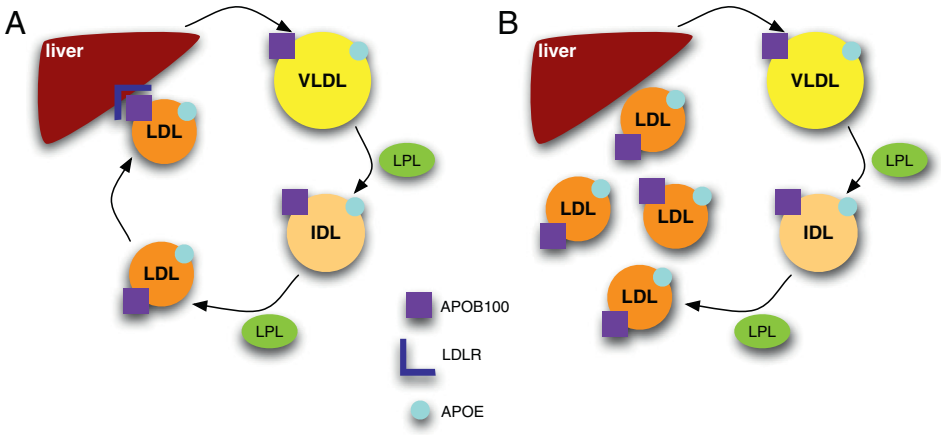

Fig. 1. Physiology of VLDL secretion and processing into LDL (A) and the pathophysiology of FH (B). (A) The liver assembles VLDL particles containing triglycerides and cholesterol, APOB100 and Apolipoprotein E (APOE). The action of peripheral tissue lipoprotein lipase (LPL) hydrolyses the triglycerides to fatty acids which are taken up by the tissues, and the VLDL particles are progressively metabolised to intermediate density lipoprotein (IDL) and then low density lipoprotein (LDL). Some of the cholesterol-rich LDL particles are bound by LDLR and non-hepatic LDL receptors (scavenger receptors) in peripheral tissues, allowing cholesterol to be transported to these tissues. The remaining LDL particles (40–60%) are cleared by the liver via binding of LDL receptor (LDLR) to APOB100. (B) Defective or missing LDLR leads to reduced hepatic clearance of LDL particles, accumulation of LDL particles in circulation, and accelerated atherosclerosis.

HeFH and HoFH in unselected populations have traditionally been quoted at 1 per 500 and 1 per million respectively, more recent data indicates that these conditions may be much more prevalent than previously recognised at 1 per 200 and 1 per 160,000–320,000 respectively [3]. FDB is also relatively common in the population at 1 per 1000 [5]. Frequently, patients with these conditions are not aware that they have hypercholesterolaemia until they present with myocardial infarction or stroke. This makes case detection and effective early treatment paramount in the management of these conditions.

In HoFH patients, lipid lowering therapy, such as with high-dose, high-potency HmG CoA reductase inhibitors ('statins'), is able to reduce the morbidity and mortality of atherosclerosis. However, present lipid lowering drugs are not potent enough to reduce LDL cholesterol levels to target. As a result, HoFH patients are still exposed to early development of cardiovascular disease, and they suffer side effects from the treatment such as myalgia and elevations in liver transaminases [6]. Other treatments, such as LDL apheresis, are temporarily effective but are extremely expensive and invasive [7]. As a result, new treatments for HoFH are sorely needed.

This review will summarise some recent developments in the field of therapy for FH. Specifically, it will concentrate on genetic approaches to FH therapy, notably APOB100 knockdown using antisense oligonucleotide and RNA interference approaches, PCSK9 knockdown to increase cell-surface LDLR expression, and splice-switching as a means of re-engineering APOB expression and function.

1. *LDLR* gene replacement therapy

Given the fact that most cases of FH are due to defective *LDLR*, the simplest approach to therapy would be to transduce a good copy of *LDLR* into hepatocytes, restoring LDLR expression and function, and thereby reducing LDL cholesterol levels. Initially, promising results were obtained in animal studies, such as in *Ldlr* knockout mice where the introduction of the human *LDLR* gene via an adenoviral vector was shown to ameliorate the hypercholesterolaemia observed in these mice after high-cholesterol feeding [8]. It was therefore natural to move on to early clinical studies, and Grossman et al. were able to demonstrate successful transduction of LDLR into the livers of 5 patients with HoFH using a retroviral vector in 1995. In one patient, there was a significant reduction in LDL cholesterol, implying restoration of LDL catabolism [9]. Despite this significant early progress, the initial promise of gene replacement therapy has not been fulfilled. The main roadblocks to gene replacement have been in obtaining efficient transduction of enough copies of the *LDLR* gene into the liver to assure sufficient restoration of LDL clearance, and concerns regarding the safety of the viral vectors used for this purpose remain. More modern vectors such as adeno-associated virus (AAV) serotype 8 may address the efficiency issue, and show promise in animal models [10]. Even if successful, however, this *LDLR* replacement strategy does not address the subset of patients who have FH due to mutations in *PCSK9*, *LDLRAP1*, or those with FDB.

2. APOB100 knockdown

APOB100 is the integral structural apolipoprotein for LDL particles. As mentioned earlier, APOB100 is assembled with triglycerides, cholesteryl esters and cholesterol into VLDL particles by the liver before secretion. Therefore, down-regulation of APOB100 expression leads to reduced VLDL assembly and secretion, and reduced LDL particle levels. This approach has been adopted by Isis Pharmaceuticals/Genentech with their antisense oligonucleotide-based drug, mipomersen. Mipomersen is a hybrid antisense oligonucleotide (ASO) which combines 2′-O-methoxyethyl RNA 'wings' with a central portion of 10 nucleotides which is based on DNA. This 'gap-mer' ASO is able to bind to the *APOB* mRNA within hepatocytes. The hybridization of the central portion's DNA to the mRNA acts as a substrate for RNase H, which cleaves RNA/DNA hybrids. In this way, the *APOB* mRNA is destroyed, leading to successful knockdown of APOB100 expression and consequent reductions in LDL cholesterol [11,12]. In clinical trials in patients with HoFH, weekly subcutaneous injections of mipomersen were able to reduce LDL cholesterol levels by ~25% [13]. The expectation is that this will translate to reduced morbidity and mortality, supported by animal studies [14]. However, mipomersen's pathway to the bedside has been beset by safety issues. The most common adverse effects were injection site reactions, elevations in liver function tests and flu-like symptoms. As a result, mipomersen has only been approved by the FDA under a Risk Evaluation Mitigation Strategy (REMS) [15]. Mipomersen has not been approved by the European authorities who cited specific concerns regarding the adverse effects, the relatively high drop-out rate from treatment, and an apparent increase in cardiovascular events in treated patients, in contradiction to expectations [16]. Lastly, mipomersen has been marketed at a cost of at least US$176,000 per annum. Given that this is a lifelong treatment, the cost will limit its appeal and restrict its use only to the most affected HoFH patients.

Small interfering RNA (siRNA) technology has also been used to knockdown *APOB* expression in non-human primates, leading to