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ETC-216

Atherosclerosis Therapy
Treatment of Acute Coronary Syndrome

AIM
ESP-24217

Complex consisting of the recombinant disulfide-linked form of the apolipoprotein A-I_{Milano} (apoA-I_M) variant (Arg^{173}→Cys) and the phospholipid 1-palmitoyl-2-oleoylphosphatidylcholine

Complex of recombinant human apolipoprotein A-I_{Milano} and 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phosphochloline

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Abstract

Atherosclerotic cardiovascular disease is the leading cause of death in the U.S. and LDL cholesterol-lowering drug therapy has been a standard treatment for years. However, because low HDL levels have been associated with an increased risk for coronary heart disease, another attractive strategy for the treatment of atherosclerosis involves raising HDL levels to increase reverse cholesterol transport (RCT). About 70% of total HDL is comprised of apolipoprotein A-I (apoA-I), a structural protein that plays a major role in RCT, thus making it a potential HDL-enhancing target. An even more attractive target is apoA-I_{Milano} (A-I_M), a molecular variant of human apoA-I that differs from wild-type apoA-I by an Arg^{173}→Cys substitution, leading to the formation of disulfide-linked homodimers (A-I_M/A-I_M), which have an improved capacity for cell cholesterol removal. Recently, a recombinant form of A-I_M/A-I_M was produced in *Escherichia coli* and complexed with a naturally occurring phospholipid (1-palmitoyl-2-oleoylphosphatidylcholine [POPC]) to mimic native HDL. The resulting synthetic HDL, referred to as ETC-216 (AIM, ESP-24217), was shown to inhibit smooth muscle cell proliferation and prevent neointima formation in rabbit models of vascular injury, as well as to inhibit in-stent stenosis in porcine coronary arteries. ETC-216 has also shown efficacy in clinical trials, where it caused significant regression of coronary atherosclerosis in patients with acute coronary syndromes. ETC-216 continues to undergo phase II development for the treatment and prevention of atherosclerosis and acute coronary syndromes.

Introduction

Atherosclerosis is a disease of medium and large arteries that results from the deposition of fatty substances, cholesterol, cellular waste products, calcium and other materials which together form a plaque in the inner wall of an artery. This plaque can partially or completely block blood flow through the artery, and its presence leads to stimulation of the artery wall to produce other substances and cause further accumulation of cells and the formation of connective tissue within the intima. Atherosclerosis can eventually lead to stroke (following plaque rupture and formation of a thrombus), coronary heart disease and/or myocardial infarction (1, 2).

Abnormally high levels of serum cholesterol is the major risk factor for atherosclerosis. The National Cholesterol Education Program (NCEP-ATP III) recommends that adults and children aged 2 years and older should maintain total blood cholesterol levels below 200 mg/dl. Low-density lipoprotein (LDL) is the major cholesterol transporter in blood, carrying 60-80% of the body's cholesterol from the liver to the rest of the body. High levels of circulating LDL can lead to cholesterol buildup on arterial walls. Thus, LDL cholesterol levels are recommended to be maintained below 150 mg/dl. High-density lipoprotein (HDL) is another cholesterol carrier that is involved in reverse cholesterol transport (RCT), by which it transports excess lipids from peripheral cells in arterial walls to the liver, where they are eliminated in the bile. Approximately 25-33% of circulating cholesterol is transported by HDL, which is considered to exert protective effects on the heart and coronary arteries. The NCEP-ATP III recommends that HDL cholesterol levels should be above 40 mg/dl to help prevent heart disease (1, 2).

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Atherosclerotic cardiovascular disease is the leading cause of death in the U.S. and developing countries. It accounts for 14,979 deaths/100,000 population every year in the U.S. and it is estimated that more than 100 million Americans have total blood cholesterol levels above 200 mg/dl, with about 40.6 million adults having levels in excess of 240 mg/dl. In an effort to prevent hypercholesterolemia and atherosclerosis, dietary and lifestyle modification are the first strategies prescribed. If these fail, pharmacotherapy is initiated. LDL cholesterol-lowering drug therapy has been a standard treatment for years, with a wide range of lipid-lowering drugs on the market and under development (1, 3).

However, another attractive strategy for the treatment of atherosclerosis involves raising HDL levels to increase RCT. Independent of LDL levels, low HDL levels have been associated with an increased risk for coronary heart disease and nearly 50% of all patients suffering from coronary artery disease have low HDL cholesterol levels. Moreover, increasing HDL cholesterol levels appears to reduce the risk of reinfarction and stroke in coronary patients (1, 4-11). A regimen that is able to decrease LDL and triglycerides and increase HDL levels therefore represents an extremely attractive approach for the treatment and prevention of atherosclerosis. However, relatively little progress has been made in the development of agents to achieve this therapeutic goal (1).

HDL is composed of lipids (cholesterol and phospholipids) and apolipoproteins (apoA-I, apoA-II and apoA-IV, apoB-48 and apoB-100, apoC-I, apoC-II and apoC-III, apoD, apoE and apoJ). An exciting target for enhancing HDL cholesterol levels is apoA-I, which accounts for approximately 70% of total HDL protein, is the most important structural protein of the carrier and plays a major role in RCT. In addition, studies have revealed a marked inverse relationship between apoA-I/HDL cholesterol levels and the risk of atherosclerotic cardiovascular disease (1, 12). ApoA-I_{Milano} (A-I_M) is the first described molecular variant of human apolipoprotein that differs from wild-type apoA-I by an $Arg^{173}\rightarrow Cys$ substitution that leads to the formation of disulfide-linked homodimers (A-I_M/A-I_M). These homodimers have an improved capacity for cell cholesterol removal. Approximately 40 carriers of A-I_M have been identified who are heterozygous for the mutation and exhibit a common phenotype. This phenotype is characterized by severe hypoalphalipoproteinemia, normal to elevated plasma LDL cholesterol levels and moderate hypertriglyceridemia, which would normally be associated with a high risk for coronary artery disease. Instead, carriers of the mutation have a low cardiovascular risk and no significant differences have been noted in carotid intima-media thickness as compared to close relatives with normal to high HDL levels (13-17).

Recently, a recombinant form of A-I_M/A-I_M was produced in *Escherichia coli* and complexed with a naturally occurring phospholipid, 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), to mimic the properties of native HDL. The resulting synthetic HDL, ETC-216 (AIM, ESP-24217), was shown to inhibit smooth muscle cell proliferation and prevent neointima formation in rabbit models of vascular injury. Thus, ETC-216 was chosen for further development as an HDL-enhancing agent for the treatment and prevention of atherosclerotic cardiovascular disease (18-20).

Pharmacological Actions

ETC-216 has been shown *in vitro* to be structurally identical to native A-I_M/A-I_M. Both display physicochemical properties that are distinct from normal apoA-I, including higher α-helical content, immobilization of aromatic side-chains in a more asymmetric and hydrophobic environment, lower tendency for self-association and multiple transitions following thermal or chemical denaturation. In addition, ETC-216 possessed an enhanced capacity as compared to apoA-I for cholesterol removal from cholesterol-loaded murine primary macrophages and hepatoma (Fu5AH) cells, although it was less effective in activating lecithin:cholesterol acyltransferase enzyme (21-23).

Several preliminary *in vivo* studies have used recombinant A-I_M complexed with various naturally occurring phospholipids to demonstrate the antiatherosclerotic effects and benefits of these synthetic HDLs. Together, the results obtained indicate the potentially superior antiatherosclerotic efficacy of the recombinant A-I_M/A-I_M dimer ETC-216.

A study using recombinant A-I_M complexed with dipalmitoylphosphatidylcholine (DPPC) reported that repeated administration significantly reduced neointimal lesions in balloon-injured ileofemoral arteries of cholesterol-fed rabbits and reduced the progression of atherosclerosis, plaque lipid content and inflammation in apoE-deficient mice. In addition, a single i.v. injection of the recombinant A-I_M complexed with DPPC (400 mg/kg) administered to cholesterol-fed apoE-deficient mice resulted in a 2-fold higher plasma cholesterol efflux-promoting capacity as compared to saline- and DPPC-treated mice. Mice administered the recombinant A-I_M complex had significantly lower lipid and macrophage content in plaques as compared to controls (24-26).

Another study using apoE-deficient mice treated with A-I_M complexed with DPPC (20 or 80 mg/kg/dose every other day from week 25 to week 30) showed that treatment with the synthetic HDL attenuated impairment of endothelium-dependent vasodilator responses. This effect was also demonstrated in an *in vitro* study using carotid arteries from normal rabbits. In this study, recombinant A-I_M complexed with dimyristoylphosphatidylcholine (DMPC), wild-type reconstituted HDL and plasma-derived HDL all significantly attenuated lysophosphatidylcholine (LPC)-induced dysfunction of endothelium-dependent vasodilatation. These results could explain the inverse relationship between HDL cholesterol and coronary heart disease (27, 28).

Pretreatment of cholesterol-fed rabbits subjected to perivascular manipulation to induce carotid artery intima thickening with recombinant A-I_M/A-I_M dimer complexed

with egg phosphatidylcholine (EPC) (100 mg EPC + 40 mg protein for 5 injections starting 5 days before perivascular manipulation) markedly inhibited carotid intimal thickening. Pretreatment was shown to inhibit proliferation of smooth muscle cells in intimal and medial tissues (29).

An *in vivo* study examined the effects of a single intramural dose of ETC-216 (14 mg/ml immediately before coronary artery stenting) on luminal narrowing in a porcine coronary artery stent overstretch model. At day 28, a significant improvement in mean lumen loss index (21 ± 22% *vs.* 43 ± 13% lumen loss) and significantly decreased intimal area (–22%) and stenosis index (0.76 ± 0.15 *vs.* 0.59 ± 0.15) and increased lumen area (+76%) were observed in ETC-216-treated animals as compared to controls. It was concluded that ETC-216 significantly inhibited injury-induced luminal narrowing by reducing intimal hyperplasia. Thus, ETC-216 may be effective in preventing restenosis following coronary stenting (30).

Clinical Studies

A multicenter, randomized, double-blind, placebo-controlled pilot trial examined the effects of ETC-216 (5 weekly i.v. infusions of 15 or 45 mg/kg) on coronary atheroma burden as measured by intravascular ultrasound (IVUS). The study randomized 57 patients with acute coronary syndromes, 47 of whom completed the protocol. Of the 10 patients who did not complete the trial, 2 receiving the high ETC-216 dose withdrew due to adverse events (*e.g.*, elevated aspartate aminotransferase [AST] with nausea, vomiting and cholelithiasis in 1 patient and chills, nausea, diaphoresis, rigors, vomiting and mild rash during infusion in another), 3 withdrew consent and 5 had IVUS studies that could not be analyzed. Minor gastrointestinal adverse events were observed in placebo and treatment groups. A significant reduction (–1.06%) in atheroma volume was observed at the end of treatment as compared to baseline in the combined ETC-216 group. The absolute reduction in atheroma volume for the combined ETC-216 group was –14.1 mm^3, representing a 4.2% decrease from baseline. In contrast, the mean percent atheroma volume increased by 0.14% on placebo. Thus, treatment with ETC-216 caused significant regression of coronary atherosclerosis in this patient population (31).

A clinical trial in healthy volunteers determined that ETC-216 was well tolerated and increased HDL levels. Based on these results, a multicenter, double-blind, randomized, pilot clinical trial evaluated the effects of ETC-216 in patients suffering from acute coronary syndrome, and found that the compound induced a significant regression of atherosclerosis. The authors suggested that the combination of functional HDL product candidates and traditional lipid regulation therapy may be effective for acute coronary syndrome (32).

ETC-216 continues to undergo phase II development for the treatment of acute coronary syndrome and atherosclerosis (33).

Source

Esperion Therapeutics, Inc. (now a division of Pfizer, Inc.) (US).

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