

Review Article

DIFFERENT CHEMICAL, BIOLOGICAL AND MOLECULAR APPROACHES FOR ANTI-HYPERLIPIDEMIC THERAPY WITH SPECIAL EMPHASIS ON ANTI-HYPERLIPIDEMIC AGENTS OF NATURAL ORIGIN

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ABSTRACT

Elevated levels of serum cholesterol leading to atherosclerosis can cause enhanced risk factors for coronary artery diseases (CAD). Reduction in serum cholesterol levels reduces the risk of CAD, substantially. Medicinal chemists all around the world have been designing, synthesizing, and evaluating a variety of new bioactive molecules for lowering lipid levels. Even so, some patients in the high risk category fail to achieve recommended cholesterol levels and to bring about regression of the already existing atherosclerotic lesions with currently available medications. Thereby, development of novel approaches to battle the world epidemics of hyperlipidemia remains relevant. In addition to existing treatments, some other recent chemical, biological and molecular approaches for the development of novel antihyperlipidemics are discussed herein. But none of these approaches are currently approved for use in humans. Several ongoing agents are in their different stages of clinical trials, in expectation of promising antihyperlipidemic drugs.

Keywords: Antihyperlipidemia, Atherosclerosis, Coronary heart diseases (CHD), Statins and Nonstatins.

INTRODUCTION

In most of the industrialized nations, hyperlipidemia and thereby atherosclerosis is the leading cause of cardiac illness and deaths [1]. About 70% of total cholesterol in the human is synthesized *de novo* and the remaining is supplied by absorption from diet (0.3-0.5 gm/day) [2]. In 1984, it was demonstrated for the first time that there exists a link between serum cholesterol levels and risk to coronary heart disease (CHD) [3]. A 1% drop in serum cholesterol reduces the risk for CHD by 2% [4]. The primary cause of CHD is atherosclerosis, a chronic disease, characterized by the accumulation of lipids and fibrous connective tissue on the arterial wall, resulting in a narrowing of the vessel lumen and ultimately hardening of the vascular system, which may lead to ischemic heart disease, myocardial infarction, and stroke [5].

Hypercholesterolemia is generally, associated with an increase in plasma concentration of LDL and VLDL. Lowering of elevated levels of LDL cholesterol can slow the progression of atherosclerotic lesions. The angiographic studies have established the fact that one of the risk factors for atherosclerotic cardiovascular disease comprises low levels of high-density lipoprotein (HDL) cholesterol concentration and shows an inverse correlation. Another study showed that a 10 mg/dl increase in HDL cholesterol were associated with a 19% decrease in coronary artery disease death and a 12% decrease in all causes of mortality [1,2].

Several other methods are presently practiced to control blood cholesterol levels. These include balance of dietary fats; HMG-CoA reductase inhibitors, bile acids sequester, fibrates, cholesterol absorption inhibitors etc. But no class of drugs is as widely prescribed or as heavily studied as those that inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase ("statins") [2]. The reasons for extensive use of statins is their favorable efficacy and safety profiles and their benefit in reducing the risk for cardiovascular events (CVEs) and death in patients with or without established cardiovascular disease (CVD). They are also the recommended first-line treatment for hyperlipidemia and for the prevention of CVD by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP-III) [6]. Pitavastatin calcium is the statin most recently approved by the US Food and Drug Administration and is the seventh statin available in the United States [7].

Although a range of synthetic drugs are available as anti-hyperlipidemic drugs, many of them do not fulfill all requirements and their numerous side effects and potential interference with drug metabolism are common. Thus, the search is on for better medicaments especially from the plant kingdom which might provide a useful source for therapy or alternatively as simple dietary adjuncts to existing therapies [2]. Many such medicinal plants have been studied in this context.

Hyperlipidemia and atherosclerosis

Different stages in progression of atherosclerosis [8].

Different stages of atherosclerosis from the normal, healthy coronary artery (stage I) until the final stage (stage V) leading to its complete blockage resulting in heart attack are described in Fig 1.

Stage I (Normal Artery)

The inner lining of the normal coronary artery is smooth and free of blockages or obstructions.

Stage II (Fatty Streak)

However, with increasing age lipids or fatty substances (cholesterol and triglycerides) are deposited as fatty streaks which are only minimally raised and do not produce any obstruction or symptoms. This is just the beginning of atheroma.

Stage III (Early Atheroma)

Further increase in built up of fatty layers, atheroma, begins to encroach the inner channel which starts interfering with the free blood flow through a coronary artery, thereby exposing the person to more risk of coronary artery disease.

Stage IV (Plaques Formation)

With fibers beginning to grow in the fatty layers of the atheroma, the blockages harden into plaques, which increase the encroachment in the inner channels of the coronary artery.

This encroachment may be up to 50% or more of its diameter and leads to obstruction sufficient to decrease the blood flow of heart muscle, even in the time of its increased need (exercise, emotional stress). This leads to elevation in blood pressure and heart rate.

Stage V (Thrombosis of ruptured plaque)

In some cases, plaques within the inner lining of the coronary artery may develop a slight crack or rupture, which stimulates the production of blood clots.

The clots also get into the crack and cause it to rise and further obstruct the channel of the artery. The supply of the blood flow to the heart muscle is substantially reduced and the patient begins to have severe and prolonged chest pain that occurs at rest. This is known as unstable angina.

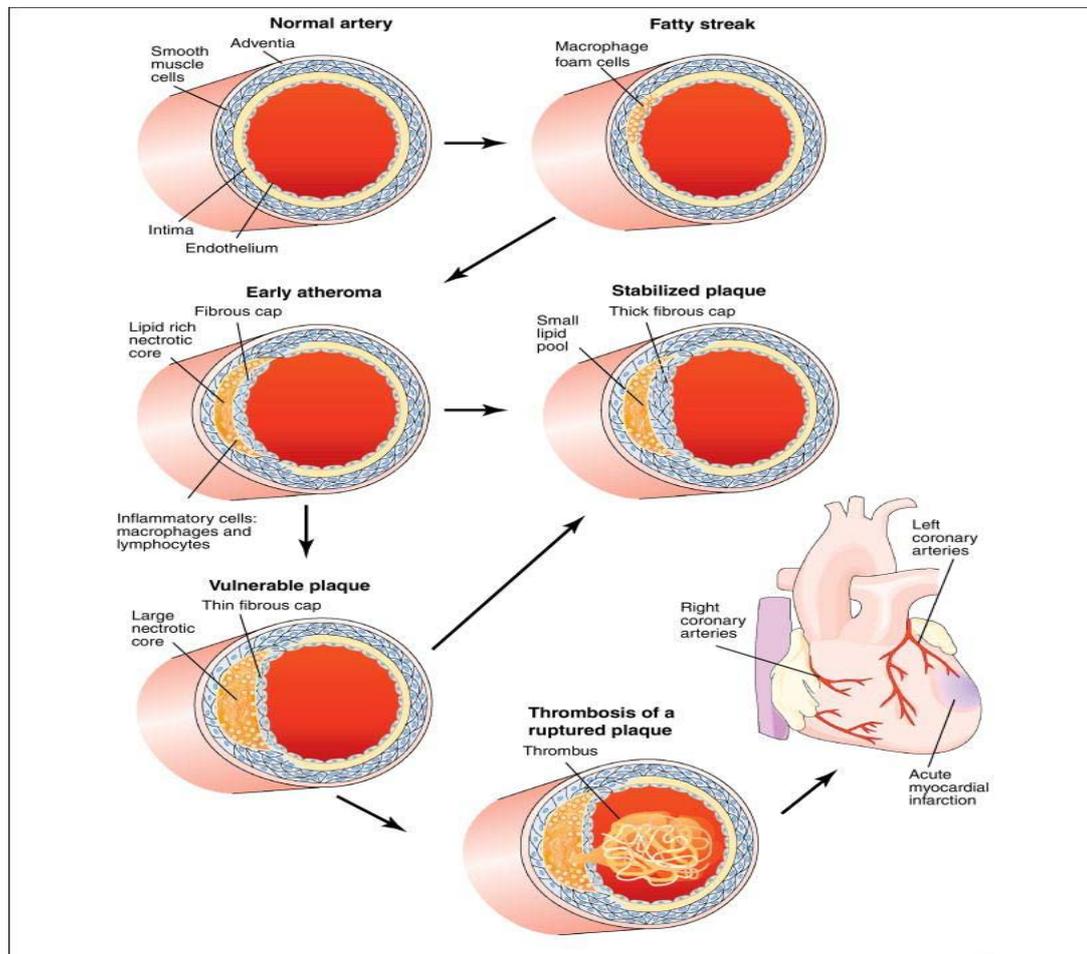


Fig. 1: Different stages in progression of atherosclerosis [8].

Existing treatment

The blood cholesterol mainly comes from two major sources:

(i) biosynthesis of cholesterol by the liver and (ii) absorption by the intestines. Both play a major role in the overall balance of cholesterol. Given this fact, to date, the conventional drugs treatment for lipid alteration has focused on the intervention of these two sources.

Statins

3-Hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase is the rate-limiting enzyme in the cholesterol biosynthetic pathway. The first HMG-CoA reductase inhibitor, compactin was discovered by a Japanese microbiologist, Akira Endo, in a fermentation broth of *Penicillium citrinum* in the 1970s, during a search for antimicrobial agents. However, due to serious animal toxicity it was eventually aborted. After that, the first generation statins lovastatin was developed by Merck Corporation [9].

Statins are definitely a revolutionary discovery of the dyslipidemia treatment as they effectively lower LDL-C and triglycerides (TG) levels with small increase in HDL-C as well [10]. Apart from this, statins have anti-inflammatory effect because they lower CRP (C-reactive protein), an acute phase reactant and prognostic marker for the inflammation responsible for development of atherosclerosis, plaque rupture and fissure formation [11]. In addition, statins have

also been touted to have several lipid-independent beneficial effects, including raised endothelial nitric oxide production, declined platelet aggregation, and decreased smooth muscle proliferation [10,12]. Although statins are effective cholesterol-lowering agents, and well-tolerated by most of the patients, common adverse effects of statins include liver enzyme and creatine kinase elevations with or without concomitant myalgia, myopathy, impaired cognitive function, nephropathies, and/or hepatic dysfunction while rhabdomyolysis is rare, but potentially fatal side effect. Myalgia, muscle aches, or cramps are the most frequent muscle related complaints. The damage to skeletal muscle may take the severe form of rhabdomyolysis, particularly in cerivastatin treated patients [12-13]. According to the recently patent literature, three new statins have progressed to clinical studies including BMS-644950, PF-03052334 and PF-03491165 [14, 15,16].

Fibrates

The fibrates, an isobutyric acid derivative, primarily activate lipoprotein lipase which is a key enzyme in the degradation of VLDL resulting in lowering of circulating TGs. This effect is exerted through PPAR- α , a gene transcription regulating receptor expressed in liver, fat and muscles. Activation of PPAR- α enhances lipoprotein lipase synthesis and β -oxidation of fatty acids [17]. Enhancement of lipoprotein lipase activity is mediated through decreased production of hepatic apo CIII, a protein component of VLDL [18]. Furthermore,

fibrates increase HDL-C and apo A-I levels by upregulating apo A-I and apo A-II and seem to enhance HDL function, including reverse cholesterol transport [10]. In this way, fibrates lower LDL-C by 10–20% reduce TG by 25–45% and increase HDL-C modestly by 10–15% [19]. Fibrates are used in accessory therapy with statins; however, clinical trials do support their use in monotherapy of hyperlipidemia. Clinically, fibrates are well tolerated. The most serious adverse effect is muscle toxicity and subsequent rhabdomyolysis [10].

Bile acid sequestrants

Bile acids are large polymeric molecules synthesized from cholesterol in the liver and transit to the intestinal lumen, where they emulsify diet fats, aiding in their absorption. Bile acids are then reabsorbed by active ileal uptake and recycled through the enterohepatic circulation. Bile acid sequestrants (BAS), such as colestipol, colestyramine and colesevelam, are anion exchange resins being similar in mechanism of binding bile acids in the intestinal lumen. They interrupt enterohepatic circulation of cholesterol-rich bile acids and increase their fecal excretion, leading to the depletion of intrahepatic cholesterol, which causes up regulation of LDLR [20]. Hepatic LDL-C uptake is thereby raised, resulting in augmented LDL particle clearance and lowering LDL-C level up to 20% [21]. BAS also interfere with the absorption of lipophilic vitamins, which is especially important in children, and cause constipation in 30% of patients [22]. Furthermore, resins can bind and inactivate polar drugs including statins, warfarin, digoxin, and folic acid [23].

Niacin

Niacin, also known as vitamin B3 or nicotinic acid, favourably affects apoB containing lipoproteins, and can reverse atherosclerosis in large doses (prescribed in doses between 1000 and 2000 mg) by lowering total cholesterol, triglycerides, atherogenic lipoproteins, such as VLDL and LDL [24]. Nicotinic acid inhibits hormone-sensitive lipase (HSL)-dependent lipolysis in adipose tissue and diacylglycerol acyltransferase 2 (DGAT2)-dependent triglyceride synthesis in hepatocytes, thereby lowering the concentration of free fatty acids in the plasma. This in turn causes decreased hepatic VLDL secretion and subsequently reduces LDL [25]. A low affinity receptor, HM74 (GPR109B), and the highly homologous high-affinity receptor, HM74A (GPR109A), encode for G-protein-coupled receptors involved in the metabolic effects of nicotinic acid and the pharmacodynamic effects of the drug may be limited by distinct haplotypes of both genes [26,27].

Specifically, niacin-induced GPR109A activation in adipose tissue inhibits hormone-sensitive lipase, subsequently declining circulating free fatty acids and hepatic VLDL production and, thus, plasma LDL-C and triglyceride levels [28]. However, more-recent clinical research testing nicotinic acid receptor agonists has suggested that other, as yet unknown, mechanisms could be involved [10].

Ezetimibe - Decrease of cholesterol absorption from diet.

Ezetimibe (SCH 58235), approved by the US Food and Drug Administration in October 2002, is the first of a new class of lipid-lowering medications, the cholesterol absorption inhibitors [29]. Ezetimibe selectively inhibits cholesterol absorption at the brush border of the small intestine by blocking the Niemann-Pick C1-like 1 (NPC1L1) protein cholesterol transporter [30]. In addition to NPC1L1 inhibition, decreased cholesterol absorption leads to a compensatory upregulation of LDL receptors on the surface of cells and an increased LDL-cholesterol uptake into cells, and thus decreased of blood LDL-cholesterol content contributes to reduction of risk for atherosclerosis and cardiovascular events [31]. Ezetimibe (10 mg/day) appears to inhibit cholesterol absorption by more than 50%; however, the principle medical benefit appears to be a reduction in LDL-cholesterol [32]. Ezetimibe monotherapy can reduce LDL-C levels by approximately 18%, as well as TG and apoB levels by around 5 and 15%, respectively. In addition, significant elevation of HDL-C has also been observed [10]. It is also indicated for use in combination with atorvastatin or simvastatin for the reduction of TC and LDL-C levels in patients with homozygous

familial hypercholesterolemia (Hoz-FH) and as monotherapy in patients with homozygous familial sitosterolemia (Hoz-FS) [33].

Omega-3 fatty acids

Omega-3 fatty acids (docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]) are polyunsaturated fatty acids that have a broad range of biological actions including hypotriglyceridemic, anti-inflammatory, anti-aggregatory and antiarrhythmic responses. Treatment of omega-3 fatty acids at pharmacological doses (3–12 g per day) has been demonstrated to reduce elevated triglyceride levels and have modest effects on non-HDL-C and apo B levels, but not decrease LDL-C levels [34]. Different mechanisms have been proposed for the effects, including reduced hepatic synthesis of triglycerides via inhibition of acyl CoA: 1,2-diacylglycerol acyltransferase, as well as decreased esterification and release of other fatty acids. Apart from this, they increase hepatic β -oxidation and upregulate fatty acid metabolism in the liver by stimulating PPARs, subsequently diminishing the availability of free fatty acid for triglyceride synthesis [35].

Recent scientific approaches

With the hope of providing additional approaches to lowering LDL-C levels, many agents which differ in actions are being investigated.

Squalene synthase/ farnesyl diphosphate farnesyltransferase (FDFT1) inhibitors

Many clinical studies have revealed that cholesterol lowering therapy with statins significantly reduces the risk of coronary heart disease. However, high-dose statins, increase the risk of myotoxicity. This toxicity is thought to result from the reduction of isoprenylated metabolites such as ubiquinones, dolichols or isoprenylated proteins in tissues [36]. Lapaquistat, previously called as TAK-475, lowered plasma cholesterol levels by blocking the squalene synthase, a key enzyme that catalyzes the conversion of farnesyl diphosphate to squalene in the cholesterol biosynthesis. Since farnesyl diphosphate is a precursor of isoprenylated metabolites (Fig. 2), they may be increased by lapaquistat. Thus, the combination of lapaquistat with statins is expected to prevent the decrease in isoprenylated metabolites by statins, which may reduce the frequency of statin-induced myopathy [37].

Most squalene synthase inhibitors (SSIs) including zaragozic acid [38], TAK-475, are structural analogs of farnesyl pyrophosphate or pre-squalene pyrophosphate, which are effective in LDL-C lowering, along with the serious adverse effects. Thus, the progressions of these agents are limited. Another one of the most promising SSIs is lapaquistat acetate experiments suggested that lapaquistat decreased LDL-C and TG in various animal models, via the up regulation of the LDLR and the decrease of apoB100 production [39].

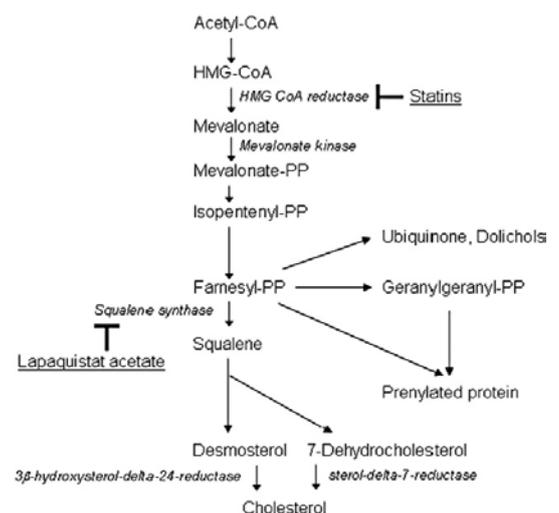


Fig. 2: Cholesterol biosynthetic pathway and its inhibitors [37].

Other SSIs, EP2302 and EP2306 have discovered which decrease cholesterol and triglyceride biosynthesis and apoB secretion, and increased LDL receptor expression and LDL uptake in HepG2 cells [40]. A novel series of squalene synthase inhibitors, 4H,6H-[2]benzoxepino[4,5-c][1,2]oxazoles have been identified which have a superior profile compared to lapaquistat acetate (TAK-475) and its active metabolite T-91485 [41]. Compound YM-75440, a propylamine derivative, has been emerged as an orally acting SSIs having potential lipid lowering action [42].

Squalene epoxidase (SQLE) and lanosterol synthase (LSS)

Squalene epoxidase and the oxidosqualene cyclase (lanosterol synthase) are responsible for the formation of lanosterol, the first sterol in the cholesterol synthesis pathway. Squalene epoxidase (SQLE) is a FAD containing enzyme located in the endoplasmic reticulum which catalyzes the epoxidation of squalene producing 2,3-oxidosqualene. Another enzyme located in the endoplasmic reticulum is oxidosqualene cyclase (lanosterol synthase, LSS), that converts 2,3-oxidosqualene to lanosterol, the initial four-ringed sterol intermediate in the cholesterol synthesis pathway [14]. Furthermore, 24(S), 25-epoxycholesterol is a ligand of LXR and enhanced synthesis of the oxysterol 24(S),25- epoxycholesterol in macrophages due to inhibition of LSS is a well known mechanism for the attenuation of foam cell formation [43]. Thus, through the dual action of LSS inhibitors (first, inhibition of lanosterol formation and second, formation of ligands for LXR), it may be possible to decrease plasma levels of LDL-cholesterol and to prevent cholesterol deposition within macrophages [44].

Microsomal transfer protein (MTP) inhibitors

Microsomal Triglyceride Transfer Protein (MTP), found in the endoplasmic reticulum of hepatocytes and enterocytes, has been identified as one of the promising targets for the treatment of dyslipidemia. MTP plays crucial role in the assembly of triglyceride rich chylomicrons in enterocytes, and VLDL in hepatocytes. Inhibition of MTP thereby leads to decrease of VLDL-C, LDL-C and TG levels [10]. It was suggested that MTP inhibitors could be helpful in treating FH. Lomitapide [AEGR-733] is a promising MTP inhibitor currently being evaluated in Phase III clinical trial in Hoz-FH. A tendency to develop fatty liver and gastrointestinal symptoms is the main adverse effect of this agent. Experience from the early phase II study and experience from a Phase III trial shows that treatment with Lomitapide appears to confirm the high effectiveness of this drug. Despite early modest gastrointestinal side effects, most patients are tolerating the drug well. So far, Lomitapide would be described as the most effective LDL-C reducing drug in Hoz-FH [45,46]. Several other MTP inhibitors are in various stages of development. Such candidates are CP-346086 [47], JTT-130 [48], SLx-4090 [49], implitapide [50], dirlotapide [51] and its analogues.

Cholesterol absorption inhibitors (ACAT inhibitors)

It is believed that acyl coenzyme A: cholesterol acyltransferase (ACAT) plays a key role in the assembly and secretion of very low density lipoprotein (VLDL) in the liver, as well as in the accumulation of cholesterol esters in macrophages and arterial vascular smooth muscle cells in atherosclerotic lesions. Accumulation of cholesterol ester causes the formation of foam cells from macrophages in the arterial walls, which is a hallmark of atherosclerotic lesions [52]. Research efforts inhibition of ACAT to reduce plasma lipid levels by inhibiting intestinal cholesterol absorption and to prevent progression of atherosclerotic lesions by inhibiting the accumulation of cholesteryl esters in macrophages. Due to these advantages, the therapeutic potential of ACAT inhibitors has been recognized for the treatment of hypercholesterolemia and atherosclerosis [53,54,55]. Two isoforms of ACAT have been recognized, ACAT1 and ACAT2. The inhibition of ACAT1 could prevent the transformation of macrophages into foam cells in the vessel wall and slow the progression of atherosclerosis and prevent the development of vulnerable plaque. In addition, inhibition of ACAT2 could reduce plasma lipid levels via regulating hepatic lipoprotein and cholesterol absorption [56]. Pactimibe [57] is a potent agent, which is purported to act as inhibitor of both ACAT1 and ACAT2. The latest discovered potent ACAT1 and ACAT2 inhibitors are K-604 [58] and pyripropene A [59], respectively.

CETP inhibitors

Cholesteryl ester transfer protein (CETP) is also called plasma lipid transfer protein that facilitates the transport of cholesteryl esters and triglycerides between the lipoproteins, leading to the enrichment of HDL with triglycerides. As a strategy of improving HDL levels, inhibition of CETP activity is being studied. Blocking CETP function results in an increase in HDL-cholesterol with a decrease in VLDL/LDL-cholesterol in parallel [60,61]. The first candidate, torcetrapib, which is a direct CETP inhibitor, significantly increases HDL cholesterol levels alone or in combination with statin [62]. Although torcetrapib significantly increases HDL-cholesterol levels, no effect was shown on coronary atheroma. In 2006, Pfizer terminated the development of torcetrapib because of its hypertensive effects and associated mortality [63]. Two novel CETP inhibitors, dalcetrapib by Roche and anacetrapib by Merck, are being developed, which in contrast to torcetrapib, do not increase blood pressure. In a phase II trial, dalcetrapib significantly reduced CETP activity and increased HDL-cholesterol levels by more than 30% [64]. Anacetrapib in monotherapy or in coadministration with atorvastatin induced substantial increase in HDL-cholesterol with significant reduction of LDL-cholesterol [65].

Antisense oligonucleotides to apo B

ApoB is required for the intracellular assembly and secretion of very low density lipoproteins (VLDL) and LDL by the liver. Thus, the number of apoB is positively correlated with the level of plasma LDL-C. Therefore, a possible afford has been taken to inhibit the formation of apoB during the process of translation from the gene into its protein product [66]. A potential approach to inhibit translation of mRNA is to block the process by using a single-strand antisense oligonucleotide (ASO) that is complementary to and will strongly hybridize to the mRNA. This will lead to the degradation of mRNA and eventually result in reduced transcription of the encoded protein. ApoB ASO targeting apoB100 protein synthesis is an attractive ASO since apoB100 is highly distributed in the liver [67]. Current research mainly focuses on apoB100 ASO, with mipomersen monotherapy. [Mipomersen is an apolipoprotein B synthesis inhibitor for lowering of LDL-C in patients who are already receiving lipid-lowering drugs, including high-dose statins.] [68].

Proprotein Convertase Subtilisin Kexin type 9 inhibition

Proprotein convertase subtilisin/kexin type 9, also known as PCSK9, is an enzyme that in humans is encoded by the PCSK9 gene. This gene encodes a proprotein convertase belonging to the proteinase K subfamily of the secretory subtilase family. The encoded protein plays a major regulatory role in cholesterol homeostasis. PCSK9 binds to the epidermal growth factor-like repeat A (EGF-A) domain of the low-density lipoprotein receptor (LDLR), inducing LDLR degradation in liver. Reduced LDLR levels result in decreased metabolism of low-density lipoproteins, which could lead to hypercholesterolemia [69].

Thus, drugs that block PCSK9 can lower circulating cholesterol. Although, the exact mechanism by which PCSK9 affects LDLR is yet to be determined, PCSK9 acts by targeting the LDLR to lysosomes in a process that involves a direct protein-protein interaction with the receptor and does not require its catalytic activity [70,71]. Interestingly, statins and ezetimibe treatment were associated with increased levels of circulating PCSK9, which possibly attenuate the therapeutic effects of them. Thus, PCSK9 is considered by many to be a highly desirable therapeutic target for the generation of novel cholesterol-lowering drugs, or in combination with statins and ezetimibe to enhance the lipid-lowering efficacy [72].

Thyromimetics

Thyroid hormone enhances the expression of the hepatic LDLR gene [73], thereby, increasing LDL clearance and decreasing plasma LDL-C levels [74]. Thyroid hormone (in particular 3,5,3'- triiodo-L-thyronine (T3) has, therefore, been tested for use as a cholesterol-lowering agent, but was associated with adverse effects on heart and bones [75]. It is known that T3 exerts its effects via four known isomorphs of the thyroid receptor (TR), TR- α -1, TR- α -2, TR- β -1 and TR- β -2. Further, TR- α plays a major role in the heart, while TR- β ,

highly expressed in the liver, controls cholesterolemia by mediating the activation of CYP7A in response to T3 [76,77]. Subsequently, selectively targeting TR- β without the cardiac complications has been developed. Currently, one such agent eprotirome (KB2115), a selective agonist of TR- β , is undergoing clinical trial [10].

Activators of peroxisome proliferator activated receptor (PPAR)

The imbalance of lipid homeostasis is manifested in hyperlipidemia and hyperlipoproteinemia which covers not only hypercholesterolemia, but also hypertriglyceridemia. The abnormal blood levels of triglycerides are not essentially associated with high levels of cholesterol; however, the treatment of hypertriglyceridemia also has secondary effects on increased cholesterol levels and vice versa. PPARs are nuclear receptors that control lipid metabolism, and play a central role in the maintenance of lipid homeostasis [78]. Three PPARs have been identified: PPAR α expressed in liver, kidney, muscle or adipose tissues; PPAR δ expressed in brain, adipose tissues and skin; and PPAR γ expressed in almost all tissues [79]. Peroxisome proliferators and fatty acids are able to activate PPAR α , which mediates the induction of peroxisomal enzymes catalyzing β -oxidation of fatty acids. PPAR α and PPAR γ are the molecular targets of a number of marketed drugs, such as the fibrates, the activators of PPAR α , and the thiazolidinediones, the activators of PPAR γ [80].

Thiazolidinediones

The lipid profile of the patients with acute complications (hypo- or hyperglycemia and diabetic ketoacidosis) is often far from normal with increased levels of VLDL, LDL and triglycerides as well as reduced levels of HDL [81,82]. Besides lowering high blood glucose levels, thiazolidinedione therapy also has beneficial effects on dyslipidemia. They selectively activate the nuclear receptor PPAR γ , and modulate the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism. Thus, the novel agents related with this moiety is being developed to improve abnormalities of lipoprotein, cholesterol or triglycerides levels in patients with type 2 diabetes. Pioglitazone with or without concomitant antidiabetic medications decreases the levels of serum triglycerides, total cholesterol and LDL-cholesterol, and increases HDL-cholesterol in patients with disorders of the lipid metabolism [83,84,85]. In both monotherapy or in combination therapy, pioglitazone appears to be associated with greater beneficial effects on lipid profile than rosiglitazone [84].

Recent scientific approaches based on natural origin

In spite of the presence of known antihyperlipidemic medications in the pharmaceutical market, many of them do not fulfill all requirements and have numerous side effects. Thus, better remedies from medicinal plants are being explored to treat hyperlipidemia.

Murraya koenigii (L.) spreng leaves and mahanimbine [86].

The dichloromethane (MKD) and ethyl acetate (MKE) extracts of *Murraya koenigii* leaves significantly reduced the body weight gain, plasma total cholesterol (TC) and triglyceride (TG) levels when given orally at a dose of 300 mg/kg/day to the high fat diet (HFD) induced obese rats for 2 weeks. The observed antiobesity and antihyperlipidemic activities of these extracts were correlated with the carbazole alkaloids present in them. Mahanimbine when given orally (30 mg/kg/day) also significantly lowered the body weight gain as well as plasma TC and TG levels. These findings demonstrate the excellent pharmacological potential of mahanimbine to prevent obesity. Thus, the antiobesity and the lipid lowering activities of the *M. koenigii* leaves have been developed as a nutraceutical for the treatment of obesity and related metabolic disorders. Mahanimbine can be used as a marker as well as biomarker compound for *M. koenigii*. It can also be taken up as a prototype for discovery of newer antiobesity agents.

Buckwheat leaf and flower (BLF) [87,88].

Buckwheat, a crop utilized throughout the world, is one of our important food sources. Besides various polyphenols, it contains

proteins with high biological value and balanced amino acid composition, fibres, vitamins B₁ and B₂, zinc, copper, manganese and selenium. Recently, attention was paid to the identification of the individual components of the phenolic fraction and their antioxidant effects. The antioxidant activity in buckwheat exhibited a statistically significant relationship with its total phenolics, as well as rutin content. Supplementation of the powdered BLF mixture that is rich in phenolic compounds and fibre was found to suppress the body weight gain and lower plasma and hepatic lipid concentrations with a simultaneous increase in faecal lipids in rats on a high-fat diet. An efficacy test of lipid-lowering action of the BLF mixture, suggests that these plant parts would be beneficial for regulation of lipid metabolism or prevention of hyperlipidemia in experimental animal models.

Vernonia anthelmintica seeds [89,90,91].

The recent study confirms the antihyperglycemic and antihyperlipidemic property of *V. anthelmintica* seeds in STZ induced diabetic rats. Administration of crude ethanolic extract of *V. anthelmintica* seeds at a dosage of 0.50 g/kg tended to bring blood glucose levels towards near normal levels. The decreased antihyperglycemic activity at higher doses could be due to reduced or no effect of the components present in the extracts at higher doses and/or the presence of other antagonistic components in the extract. But the extract did not produce any hypoglycemic effect in normal rats.

Hence the ethanolic extract may be considered to have good antihyperglycemic active principle(s) without causing any hypoglycemic effect unlike insulin and other synthetic drugs. Administration of the active fraction (100 mg/kg body weight) for 45 days resulted in significant reduction in plasma glucose, HbA1C, cholesterol, triglycerides, LDL, VLDL, free fatty acids, phospholipids and HMG-CoA reductase in STZ diabetic rats.

Ulva pertusa (Chlorophyta) [92,93].

The green alga, *Ulva pertusa*, is an important food source in many parts of the world. Algal sulfated polysaccharides have been reported to possess diverse biological activity of potential medicinal value. In a study, high sulfate content ulvan (HU) was prepared with sulfur trioxide/N,N-dimethylformamide (SO₃-DMF) in formamide, and the antihyperlipidemic activity of natural ulvan (U) and HU in mice was determined. The antihyperlipidemic activity of HU-fed 250 mg/kg was the strongest, compared to natural ulvan fed group, HU exhibited stronger antihyperlipidemic activity than U. It was likely that the sulfate content had significant effect on the antihyperlipidemic activity.

Pyrus bioessieriana buhse leaf [94].

The wild pear, *Pyrus bioessieriana* Buhse is a species of pear that belongs to the plant family Rosaceae. *Pyrus bioessieriana* Buhse grows in northern Iran and Turkmenistan. A methanolic extract of *Pyrus bioessieriana* Buhse at doses of 500 and 1000 mg/kg can significantly reduce blood glucose and lipid levels and increases antioxidant status in rats with alloxan-induced hyperglycaemia. These effects are relatively long-lasting.

Ficus religiosa [95].

Extensive clinical and experimental studies have shown that the dietary fiber influences the lipid level of the blood and tissues to different extent, depending on their nature and quantity. *F. religiosa* fruits, being rich in fiber were evaluated for their hypolipidemic activity in male albino rats. The animals were fed with a semi-synthetic diet containing hydrogenated oils to induce hyperlipidemia. The fruit powder was incorporated in the animal diet, so as to provide 10% of dietary fiber. Treatment with the fruit fiber diet showed significant hypolipidemic effect, indicated by reduced level of serum cholesterol and phospholipids, and liver total lipids and cholesterol. The stem-bark of *F. religiosa* has shown its ameliorative effect against hyperlipidemia associated with diabetes mellitus. Although detailed studies are lacking, future work may produce interesting results and provide a potential therapeutic agent from *F. religiosa* for the treatment of hyperlipidemia.

***Cassia auriculata* flowers** [96,97].

The flower extract of *Cassia auriculata*, herb has been used traditionally in India for medicinal purposes. The plant has been reported to treat hyperglycemia and associated hyperlipidemia. Hyperlipidemia and oxidative stress are known to accelerate coronary artery disease and progression of atherosclerotic lesions. The work was undertaken to investigate the possible antihyperlipidemic and antioxidative effect of *C. auriculata* flower on triton WR 1339 induced hyperlipidemic rats without any known adverse effect.

Diosmin: A citrus flavonoid [98].

A very recent study was hypothesized to evaluate the antihyperlipidemic effect of diosmin (DS) on lipid metabolism in experimental diabetic rats. The study suggested that DS could potentially ameliorate lipid abnormalities in experimental diabetes. The concentrations of plasma lipids (cholesterol, TGs, FFAs and PLs) were increased in diabetic rats as compared to the normal control rats. Oral treatment with DS significantly ($p < 0.05$) reduced the concentrations of plasma lipids (cholesterol, TGs, FFAs and PLs). The activity of HMG-CoA reductase was enhanced (decreased HMG-CoA/mevalonate ratio indicates increased activity of the enzyme) significantly ($p < 0.05$) in liver and kidney of diabetic rats. Treatment with DS to diabetic rats significantly ($p < 0.05$) decreased the activity of HMG-CoA reductase in these tissues in comparison to diabetic control rats. Thus, administration of DS to STZ-NA-induced diabetic rats altered the plasma and tissue levels of lipids and lipid metabolizing enzymes to near normal levels. It can be stated, that the DS has beneficial effects, in the prevention and controlling of dyslipidemia associated with diabetic complications.

***Ichnocarpus frutescens* leaves** [99].

Antihyperlipidemic effects of the polyphenolic extract of *I. frutescens* leaves was evaluated in alloxan-induced diabetic rats. Administration of extract (300 mg/kg for 21 days) showed significant decrease in hepatic HMG-CoA reductase activity of treated animals. No significant effects were found in the normoglycemic rats. Polyphenolic extract exhibited significant hypolipidemic effect as evident from correction of hyperlipidemic indicators (TC, TGs, VLDL, HDL and LDL). Oral administration of polyphenolic extract (100 mg/kg) significantly enhanced the release of lipoprotein lipase enzyme significantly.

***Solanum surattense* leaves** [100].

S. surattense leaf extract (family: Solanaceae, Synonym: *Solanum xanthocarpum*) (Indian night shade) markedly reduced dyslipidemia and hyperglycemia in Streptozotocine-induced diabetic rats. The hypolipidemic effect was due to the presence of phytochemicals such as saponins, flavanoids, phenolic compounds, glycosides and triterpenoids in the leaf extract which is line with several authors.

***Bauhinia variegata* (Linn.)** [2].

A most recent study concluded that butanol extract of *Bauhinia variegata* (Linn.) in Triton WR-1339 induced hyperlipidemic rats not only have resulted in significant reduction in cholesterol, triglyceride, LDL, VLDL level but also increased the HDL level at a reduced dose level.

Mogrosides extract from *Siraitia grosvenori* [101].

Fruits of *Siraitia grosvenori* Swingle have been used for thousands of years as a folk medicine for the treatment of lung congestion, colds, and sore throats. The main effective components of the fruit of this plant are triterpene glycosides, known as mogrosides. Of these compounds, 11-oxo-mogroside V and mogroside V exhibit a strong effect for oxidative modification of low-density lipoprotein. There is a strong evidence that lipid peroxidation plays a role in the production of free radicals and oxidative stress during diabetes. And poor glycemic control has been associated with the depletion of antioxidant capacity and hyperlipidemia. Thus, administration of the extract may be helpful in the prevention of diabetic complications associated with oxidative stress and hyperlipidemia.

Cynodon dactylon [102].

Cynodon dactylon Pers. (Family: Graminae, Durba in Bengali, Dhuh in Hindi, Bermuda grass in English) is a creeping grass found in warm climates all over the world. Concurrent administration of *C. dactylon* extract caused a significant decrease in the concentrations of serum TC, LDL, HDL, VLDL TGs when compared with cholesterol fed control rats. The mechanism by which *C. dactylon* extract lowered the serum TG concentration could be either by decreasing VLDL synthesis, by channeling VLDL through pathways other than to LDL, or an increase in lipoprotein lipase activity. Phytochemical studies of this plant have shown the presence of glycosides, flavonoids, alkaloids, tannins, and saponins. The hypolipidemic effect might be due to individual or synergistic action of these components, possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues. Alternatively, the components might exert a modulatory influence on lipogenic enzymes or by inhibition of cholesterol absorption.

CONCLUSION

It is clear that more than 70% of the body's cholesterol is derived from the de novo cholesterol biosynthesis. Thereby, the inhibition of de novo cholesterol biosynthesis by statins is currently the most effective therapeutic approach to reduce plasma LDL-C. Statins, by the opinion of some healthcare providers, are being prescribed also to the healthy population, to lower the risk of cardiovascular disease development. However, such recommendations are questionable because muscular side effects and drug interactions of statins are now better understood. In spite of several adverse effects, statins have blockbuster fame, and the novel hypolipidemic drugs have a task difficult to achieve because their hypolipidemic effect should be better or at least comparable to statins and in addition, toxicity, drug interactions and side effects should be minimal. Two examples presented in this review, the development of CETP inhibitor torcetrapib and the squalene synthase FDFT1 inhibitor lapaquistat were both terminated after clinical studies. Torcetrapib was terminated due to its hypertensive effects, leading to excess mortality while lapaquistat was discontinued due to hepatotoxicity. However, development of additional cholesterol-lowering agents with mechanisms of action distinct from statins will probably be necessary to achieve cholesterol target levels in many individuals. This review discusses the benefits and pitfalls of different groups of non-statin hypolipidemics. We hope that by presenting the state-of-the-art knowledge regarding the non-statin hypolipidemics and their potential novel targets, this review will support the notion that despite the success of statins, efforts in novel hypolipidemic approaches should be persuaded. Medicinal chemists all around the world have been designing, synthesizing, and evaluating a variety of new molecules for antihyperlipidaemic activity.

Despite the plethora of research data available on obesity, it still remains, largely, an unsolved medical problem. Phytochemicals identified from traditional medicinal plants present an exciting opportunity for the development of newer therapeutics for the treatment of obesity and other metabolic diseases. The potential of natural products for the treatment of obesity is still largely unexplored and might be an excellent alternative strategy for the development of safe and effective antiobesity drugs.

Following problems still need to be solved in the drug therapy of Hyperlipidemia.

- The most widely used 'statins' also suffer from limitations like, intolerance and adverse effects, partial effectiveness in lowering of cholesterol level and finally the 'cost'.
- Drugs are needed to be discovered, that will be able to block the stimuli causing the formation of an atherosclerotic lesion.
- Drugs are needed to be developed, that will be able to bring about regression of the already existing atherosclerotic lesions.
- Furthermore, New drugs are required to cover the hitherto untreatable cases of Type II hyperlipidemia, wherein drugs like

clofibrate, nicotinic acid, d-thyroxin, etc. are used without much success.

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