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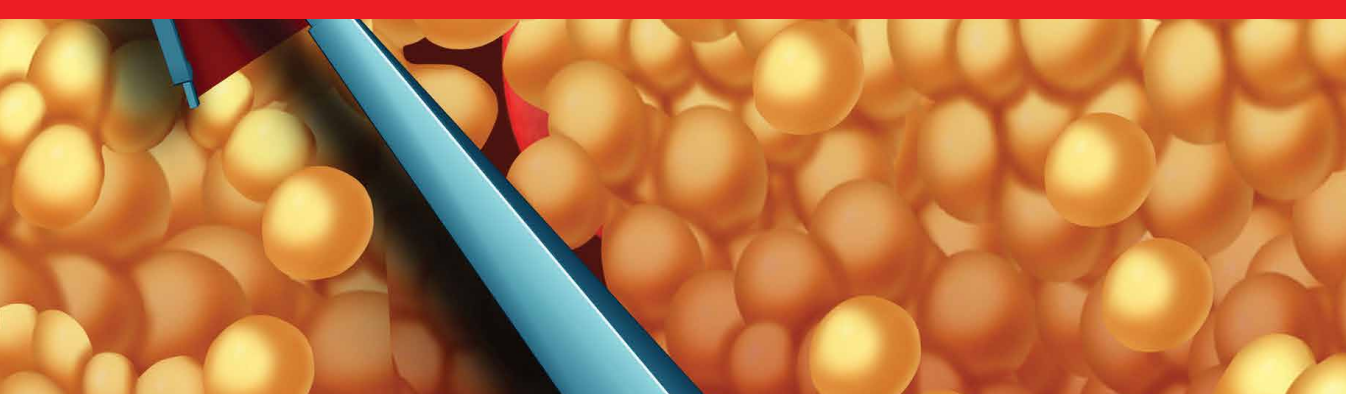
Statins

From Lipid-Lowering Benefits to Pleiotropic Effects

Edited by Donghui Liu



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Meet the editor

Dr. Donghui Liu is a senior cardiologist, geriatrician, and researcher in cardiovascular disease and geriatrics. He obtained his Ph.D. from the Physiology and Pathophysiology Department, at Peking University, China, in 2011. From 2011 to 2018, he worked in the Department of Cardiology at the cardiovascular hospital affiliated with Xiamen University, China. He currently holds the post of Associate Director in the Department of Geriatrics, National Key Clinic Specialty, Guangzhou First People's Hospital, School of Medicine, South China University of Technology. His general research interests are in the area of dyslipidemia, atherosclerotic cardiovascular disease (ASCVD), heart failure, metabolomics and organ rehabilitation in elderly patients.

Contents

Preface

Millions of patients with dyslipidemia and atherosclerotic cardiovascular disease (ASCVD) have benefited from statins, which have been used in clinical practice for almost four decades. Although the status of statins has been challenged by many novel types of non-statin lipid-lowering drugs in recent years, statins remain the primary medicine for dyslipidemia and ASCVD patients. Furthermore, the pleiotropic effects of statins are increasingly found and confirmed in gut microbiota, cancer, COVID-19, Alzheimer's disease, and motor disorders. However, alongside their widespread use, the adverse effects of statins are also emerging, such as liver injury, muscle symptoms, new-onset diabetes, etc. This book provides a comprehensive overview, based on the latest experimental and clinical evidence, of the current state of statins in cardiovascular diseases, their protective mechanisms, pleiotropic actions and adverse effects.

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Section 1

Statins' Effects on Cardiovascular System

Chapter 1

The Role of Statins in ASCVD

Cong Lu, Lu Fang, Yujie Zhu, Lemin Zheng and Donghui Liu

Abstract

Statins are comprehensive lipid-lowering agents, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. As an effective cholesterol-lowering drug, statins inhibit a key step in the cholesterol biosynthesis pathway and have made outstanding contributions to the prevention and treatment of atherosclerotic cardiovascular disease (ASCVD). The mechanism is to competitively inhibit the endogenous cholesterol synthesis rate-limiting enzyme HMG-CoA reductase, block the intracellular hydroxy valerate metabolic pathway, and reduce intracellular cholesterol synthesis. Additionally, these actions also increase the number and activity of low-density lipoprotein (LDL) receptors on the cell membrane surface and promote plasma cholesterol clearance. Therefore, statins can reduce total cholesterol and LDL levels and reduce triglycerides (TG) to a certain extent and increase high-density lipoprotein (HDL). In addition to lipid regulation, statins may also treat ASCVD by improving endothelial function, Inhibiting inflammation, and stabilizing atherosclerotic plaque. This review summarizes the fundamental roles of statins in ASCVD.

Keywords: statins, atherosclerotic cardiovascular disease (ASCVD)

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide [1, 2]. With the aging of the population and the increase in cardiovascular risk factors, the morbidity and mortality of CVD continue to increase, and the mortality of ASCVD, mainly ischemic heart disease, and ischemic stroke, has increased significantly [3]. ASCVD refers to clinically diagnosed atherosclerotic diseases, including acute coronary syndrome, stable coronary heart disease, revascularization, ischemic cardiomyopathy, ischemic stroke, transient ischemic attack, and peripheral atherosclerotic diseases. ASCVD is one of the most common clinical diseases [4].

The main risk factors for ASCVD include hypertension, dyslipidemia, and diabetes mellitus [5]. Hypertension is the leading risk factor for ASCVD morbidity and increased mortality, with approximately 50% of cardiovascular morbidity and 20% of cardiovascular mortality attributable to hypertension [6]. It is worth noting that the early prevention and control of hypertension are very important to reduce the long-term risk of ASCVD. The levels of low-density lipoprotein cholesterol (LDL-C) that were most closely associated with ASCVD were significantly elevated in the Chinese population (8.1% ≥4.14 mmol/L, 26.3% ≥3.4 mmol/L), and only 39% had an ideal level of LDL-C (≤2.6 mmol/L) [7]. Diabetes is an independent risk factor for ASCVD [8]. Once ASCVD occurs in diabetic patients, the lesions are complex, and

the prognosis is poor. Recently, domestic and foreign guidelines have listed diabetic patients as high-risk groups for ASCVD. Lifestyle interventions (weight loss, diet, and exercise) are the basis for reducing the risk of ASCVD [9], but further pharmacological interventions are needed to achieve optimal lipid control and reduce cardiovascular residual risk. Statins are currently recognized by the medical community as the most powerful drug for lowering cholesterol and significantly reducing the risk of ASCVD.

2. Classification of statins

Statins are fungus-derived molecules that have played an important role in the field of cardiovascular therapy since their discovery in the 1970s [10, 11]. Seven statins are commonly used: atorvastatin, rosuvastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and pitavastatin. Due to the different sensitivities of patients to different statins, it is necessary to make drug adjustments in the clinical application according to the range of blood lipid reduction, adverse reactions, liver and kidney function, blood glucose, and blood lipid levels of patients and then carry out statin intervention therapy after designing an appropriate treatment plan. Despite differences in pharmacokinetics and lipophilicity, the biological activities of different statins are similar, making them a fairly homogeneous class of drugs [12]. Among all statins, lovastatin, simvastatin, fluvastatin, and pitavastatin are fat-soluble statins, pravastatin, and rosuvastatin are water-soluble statins, and atorvastatin is a fat-water-soluble statin [13]. The lipid bilayer structure of the animal cell membrane makes it more difficult for water-soluble statins to enter cells, but the transporter on the surface of the liver cell membrane can selectively transport water-soluble statins into cells. Therefore, in the treatment of ASCVD, water-soluble statins can selectively inhibit liver cholesterol synthesis and thus have a low effect on cholesterol synthesis in other parts of the heart, brain, and so on, which not only effectively reduces serum cholesterol levels but also avoids the occurrence of adverse reactions in extrahepatic tissues.

Depending on the magnitude of the reduction in plasma LDL-C levels, statins can be divided into three categories: strong-efficiency statins, medium-efficiency statins, and low-efficiency statins. The disturbance of plasma lipoprotein levels, especially the abnormal increase of LDL-C concentration, plays a major role in the development of ASCVD. Therefore, the efficiency of removing excess LDL-C in plasma has become an indicator to evaluate the effectiveness of statins in the treatment of ASCVD. Atorvastatin is a powerful statin with a long effect that can be taken at any time every day. This statin is mainly metabolized by the liver drug enzyme CYP3A4 and has many interactions with other drugs, so attention should be given to the combination of drugs [14]. Rosuvastatin is also a powerful and long-acting statin that can be taken at any time. It is mainly excreted by feces, partially excreted by the kidney, and rarely metabolized by the liver. It has few interactions with other drugs and has high safety when combined with drugs [15]. Pitavastatin is a medium-efficiency statin, which is the lowest dose of statin. Pitavastatin is mainly excreted by feces and has fewer interactions with other drugs, fewer side effects, and minimal influence on blood sugar [16, 17]. Simvastatin is metabolized through the liver drug enzyme CYP3A4. It has more interactions with other drugs, and the action time is short. It should be taken before bed to maximize the lipid-lowering effect [18, 19]. Pravastatin is a medium-efficiency and short-acting statin that is not

metabolized by liver drug enzymes. It has less interaction with other drugs, fewer adverse reactions, and less influence on blood sugar [20, 21]. In the treatment of different diseases and different stages of the same disease, the appropriate intensity of statins can not only ensure effective control of the disease but also reduce the toxic side effects of treatment to a minimum.

3. The role of statins in ASCVD

3.1 Lipid regulating effect

Plasma LDL-C levels are closely related to the development of ASCVD. Therefore, pharmacological lipid-lowering therapy has become a reasonable method to prevent and treat ASCVD. Statins are mainly used to lower cholesterol levels in serum, liver, and aorta and to lower very low-density lipoprotein cholesterol (VLDL-C) and LDL-C levels. Different statins' absorption, excretion, and solubility vary, but they all reduce serum LDL in a nonlinear, dose-dependent manner [22, 23]. The role of statins includes regulating blood lipids and not regulating blood lipids. The main effect of regulating blood lipids is to reduce the levels of LDL-C and TG, but the effect of lowering TG is weak, while the level of high-density lipoprotein cholesterol (HDL-C) is increased. The nonregulating effects of blood lipids include improving vascular endothelial function, inhibiting the proliferation and migration of vascular smooth muscle cells, antioxidation, anti-inflammatory, inhibiting platelet aggregation, and anti-thrombotic effects conducive to preventing the formation of atherosclerosis or stabilizing and reducing atherosclerotic plaques. In hepatocytes, statins competitively inhibit the catalytic action of HMG-CoA reductase, resulting in decreased intracellular cholesterol levels and increased expression of LDL receptors on the cell membrane surface [24]. Therefore, statins reduce plasma LDL-C concentrations by inducing increased uptake of circulating LDL-C in liver cells [25]. In a short-term trial of 76,000 people using statins to lower LDL-C levels, the decrease in LDL-C levels increased year by year, from 11% in the first year to 24% in the second and 33% in the fifth year [26]. Therefore, the longer the statin treatment, the greater the decrease in LDL and the lower the risk of ASCVD.

In addition to high plasma LDL-C concentrations, low levels of HDL-C and high levels of TG are also risk factors for ASCVD, and ASCVD can also be prevented by improving plasma HDL-C and TG levels. Statins also play a role in managing TG and HDL-C levels, and they affect these lipoproteins to varying degrees [27]. Statins can increase the expression of LDL receptors on the membrane surface of liver cells, which not only accelerates the uptake of circulating LDL but also increases the uptake of TG-rich lipoproteins such as VLDL-C and intermediate-density lipoprotein (IDL), thereby reducing plasma TG levels [28]. The lipid-regulating effect of statins also includes an increase in HDL levels [29, 30]. Statins generally raise HDL-C levels by 10–15% [31]. Statins increase the expression of genes involved in HDL metabolism such as apoA-I. Cholesterol ester transfer protein (CETP) is the gene encoding plasma cholesterol ester transfer protein, which mainly mediates the reverse transport process of cholesterol. Statins inhibit CEPT gene expression and plasma activity [32, 33], thereby reducing HDL-C transfer mediated by CETP [34]. Therefore, statins can not only reduce plasma LDL levels but also play a role in lipid regulation by lowering TG levels and increasing HDL levels, which can reduce the risk factors for ASCVD to a large extent.

3.2 Improve endothelial function

Lowering plasma LDL-C levels is the most significant clinical benefit of statin therapy, but more evidence suggests that statins improve vascular function in a variety of ways in addition to lowering plasma LDL-C. In a clinical trial on cerebrovascular risk, LDL-C levels dropped slightly, but there was a significant reduction in cerebrovascular risk [35]. Endothelial dysfunction is the initial step in the development of ASCVD, accompanied by the aggregation of monocytes and macrophages. Statins can reduce the adhesion of monocytes and macrophages to endothelial cells (ECs) [36], which reduces the formation of foam cells and the release of inflammatory factors, ultimately inhibiting vascular inflammation and plaque formation. Pretreatment with atorvastatin has been shown to reduce the adhesion of U937 monocytes to interleukin-1β-activated endothelial cells [37]. Therefore, the inhibitory effect of statins on endothelial cell adhesion is also an important target for the clinical treatment of ASCVD.

Another manifestation of endothelial dysfunction is reduced nitric oxide (NO) biosynthesis [38–40]. NO is a well-known vasodilator and an important regulator of vascular tone, platelet aggregation, and vascular smooth muscle cell (SMC) proliferation. In ECs, NO is produced by endothelial nitric oxide synthase (eNOS). NO-mediated vasodilation is impaired in patients with ASCVD [41]. Statins have been shown to have beneficial effects on vascular tone. Statin therapy improves endothelial function by increasing NO production and utilization. In a clinical trial, fluvastatin was used to treat patients with hypercholesterolemia, resulting in increased bioavailability of NO and significant improvement in endothelium-dependent vasodilation [42]. Statins can restore NO bioavailability in a variety of ways. Statins can increase eNOS levels in ECs by stabilizing eNOS mRNA and preventing its interaction with inhibitory proteins [43]. Statins increase eNOS activity by enhancing phosphorylated inositol-3 kinase-mediated Akt [44] phosphorylation and heat shock protein 90 [45] tyrosine phosphorylation. Statins prevent the reduction in eNOS by reducing hypoxia and inflammation [46]. Statins can increase the expression of tetrahydrotrexate (BH4) by upregulating the mRNA level of guanosine 5c-triphosphate cyclic hydrolase I and promote eNOS to preferentially generate NO instead of superoxide anion [47]. In addition, statins can protect BH4 by reducing vascular oxidative stress by inhibiting the production of superoxide anions. Therefore, statins can promote NO bioavailability and improve endothelial function by increasing eNOS levels and activity.

3.3 Suppression of inflammation

Inflammation is an important pathophysiological mechanism in the occurrence and development of ASCVD and plays a role in all stages of atherosclerosis [5, 48]. In early endothelial injury, ECs are activated to maintain and enhance local inflammation and the development of atherosclerotic lesions by regulating the expression of cytokines, chemokines, and leukocyte adhesion molecules [49]. Therefore, blocking key proinflammatory mechanisms is beneficial to the treatment of ASCVD. The anti-inflammatory effects of statins have been demonstrated in several clinical trials and studies [50, 51]. Statins inhibit the production and release of proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin [52]. Other studies indicate that statins play a direct anti-inflammatory role by interfering with endothelial cell adhesion and cross-endothelial migration of white blood cells to the site of inflammation [53]. In addition, statins can limit the occurrence and development of

ASCVD by reducing the production of reactive oxygen species (ROS) and inhibiting the formation of oxidized low-density lipoprotein (ox-LDL) and foam cells.

C-reactive protein (CRP) is a nonspecific inflammatory marker that is stimulated by inflammatory cytokines in the liver. In the early stage of ASCVD, macrophages accumulate at the damaged endothelium, release a large amount of TNF-α and IL-6, and induce the liver to synthesize a large amount of CRP [54]. CRP can cause vascular endothelial cell (VEC) damage and prevent the repair and proliferation of VECs. Numerous studies have shown that statins reduce inflammation by lowering the levels of CRP [55, 56]. Although CRP and LDL can coexist in atherosclerotic plaques [57], there is no correlation between the CRP-lowering effect and the lipid-lowering effect of statins. In a clinical study of statins, atorvastatin reduced hepatogenic acute phase reactant CRP and serum amyloid A in patients with hypercholesterolemia [58]. In addition, statins regulate the expression of several proinflammatory and atherosclerotic cytokines and the formation of ROS by regulating the GTP-binding protein pathway [59].

3.4 Stabilization of atherosclerotic plaques

The occurrence of ASCVD is a slow process that is characterized by lesions of affected arteries starting from the intima and the subsequent presence of a variety of lesions, including local plaque caused by the accumulation of lipid and complex sugars, fibrous tissue hyperplasia and calcareous deposits, as well as a progressive degeneration of the arterial middle layer. Secondary lesions include intraplaque bleeding, plaque rupture, and local thrombosis [60, 61]. Therefore, inhibiting the development of atherosclerotic plaque and stabilizing the plaques that have formed are particularly important in the treatment of ASCVD. Some early clinical studies have shown that statin therapy can slow plaque progression [62, 63], which makes the effect of statins even richer. By reducing the release of proinflammatory cytokines, statins play an anti-inflammatory role while reducing the recruitment of monocytes, thus inhibiting plaque progression. In addition, statins inhibit the production of ROS and ox-LDL and reduce the number of foam cells formed by macrophage phagocytosis of ox-LDL, which also helps to slow plaque progression.

Atherosclerotic plaques can be divided into noncalcified plaques and densely calcified plaques. The larger the proportion of densely calcified plaque, the better the stability of the plaque. Proinflammatory M1 and anti-inflammatory M2 macrophages play a role in forming these two types of plaques. Studies have shown that statins can promote the transformation of the macrophage phenotype from M1 to M2 near plaques, which promotes the improvement of plaque stability [64]. The thickness of the fibrous cap is also an indicator of plaque stability, and a thin fibrous cap represents vulnerable plaque. Statins can improve plaque stability by increasing the thickness of the fibrous cap [65]. Therefore, statins can improve plaque stability by promoting the phenotypic transformation of macrophages and increasing the thickness of the fibrous cap, thus reducing the risk of subsequent cardiovascular obstruction caused by plaque rupture.

4. Conclusion and perspectives

Statins play multiple roles in the prevention and treatment of ASCVD. Statins improve plasma cholesterol levels by regulating the number of LDL receptors on

the membrane surface of liver cells and the expression of genes related to HDL metabolism. Statins can repair endothelial function by inhibiting mononuclear and macrophage adhesion to ECs and improving the bioavailability of NO. Statins inhibit inflammation by inhibiting the production of pro-inflammatory factors, ROS and CRP. Finally, statins limit plaque progression by inhibiting inflammation, promoting macrophage phenotypic transformation, and fibrous cap thickening.

In recent years, with the gradual deepening of research on the pharmacological action and mechanism of statins, the high efficiency of statins has attracted more attention. However, possible disadvantages have gradually emerged with the widespread use of statins. In addition to the financial burden of long-term use of high-dose statins, there are also very serious safety concerns, such as statin-associated muscle symptoms, new-onset type 2 diabetes, cognitive, renal, and hepatic dysfunctions, interstitial lung disease, and other reactions. These adverse reactions will greatly limit their clinical application. Therefore, doctors should evaluate the advantages and disadvantages of treatment and use methods such as reduction and intermittent use of statins, conversion between statins, replacement of nonstatin lipid-lowering drugs, the combination of statins and lipid-lowering drugs, and combination of protective drugs to reduce the adverse consequences of statin therapy. It is also hoped that a more complete treatment strategy will emerge in the future so that the risk of statin therapy can be minimized or there may even be no risk.

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Conflict of interest

The author declares no conflict of interest.

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Chapter 2

Beyond Cholesterol Reduction: Statin Pleiotropy and Peripheral Arterial Disease

Ashley Penton, Kelly A. Langert, Kristopher Maier and Vivian Gahtan

Abstract

Lower extremity peripheral arterial disease (PAD) is the atherosclerotic obstruction of the lower extremity arteries that can lead to walking impairment, non-healing open wounds, gangrene or limb loss. It is estimated that PAD affects greater than 200 million people worldwide and is associated with advanced age, tobacco use, diabetes, hypertension, and hypercholesterolemia. Initial management of PAD involves risk factor modification and pharmacologic strategies, including the implementation of statin therapy. Statins, the most commonly used cholesterol lowering medications, also have beneficial pleiotropic (cholesterol independent) effects including improved patency rates from vascular reconstruction, decreased risk of stroke, myocardial infarction and improved survival. In this chapter, we will discuss the relevant clinical trials, prospective observation and retrospective studies that exemplify the effect of statins on PAD. We will then focus on statin's cellular effects on endothelial and vascular smooth muscle cell function by examining effects on plaque progression, intimal hyperplasia, re-endothelialization, and angiogenesis/arteriogenesis.

Keywords: peripheral arterial disease, statin, pleiotropic effects, plaque stability, intimal hyperplasia, reendothelialization, angiogenesis, arteriogenesis

1. Introduction

Peripheral arterial disease (PAD) is the atherosclerotic occlusion of the lower extremities that can lead to walking impairment, gangrene, limb loss and even death. An estimated 8–12 million Americans and greater than 200 million people worldwide are affected by PAD, with prevalence being approximately 10% in patients greater than 60–70 years old [1–4]. To date, medical management includes behavioral modifications to mitigate risk factors (smoking cessation, control of hypertension, diabetes mellitus and hyperlipidemia) and treatment with medical therapies (statins, antiplatelet drugs, antihypertensives and glucose control). In addition, exercise programs are used to increase cardiovascular health and functional performance [1, 5]. Surgical or endovascular techniques are typically reserved for patients requiring arterial revascularization secondary to threatened limb viability [1].

Statins, or 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, were initially implemented in the management of PAD for their lipid lowering mechanism. However, statins have demonstrated beneficial cholesterol independent, or "pleiotropic," effects that reduce cardiovascular events, improve symptoms, graft patency, limb salvage and reduce surgical mortality [6, 7]. The pleiotropic effects are widely understood to be mediated by the ability of statins to inhibit HMG-CoA reductase, the rate-limiting enzyme of the cholesterol biosynthesis pathway (**Figure 1**) [8, 9]. Besides reducing cholesterol levels, inhibition of HMG-CoA reductase prevents the conversion of HMG-CoA to mevalonate, which limits the available pool of farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) isoprenoids. FPP and GGPP serve as important lipid posttranslational modifications of a variety of proteins, including small guanosine triphosphate (GTP) binding protein Ras, Rho and other Ras-like proteins, including Rab, Rac, Ral, and Rap [9]. Statins are believed to exert many of their pleiotropic effects through the downstream inhibition of FPP and GGPP, which disrupts the posttranslational modification and normal functioning of Ras and RhoA.

RhoA and Ras are GTP binding proteins that interact with downstream targets to elicit a variety of cellular responses [10]. In 1996, Rho-kinase (Rho-kinase-alpha/ROCK2 and Rho-kinase-B/ROCK1) was identified as the effector of RhoA [11]. Together, RhoA and Rho-kinase control multiple cell functions, including adhesion, proliferation, migration and calcium sensitivity of the contractile proteins [12, 13]. Specifically in vascular smooth muscle cells (VSMCs), Rho-kinase phosphorylates both myosin light chain (MLC) and myosin phosphatase, target subunit 1 (MYPT1). The phosphorylation of MYPT1 leads to inactivation of myosin phosphatase promoting MLC-induced vascular contraction and VSMC migration [12, 14]. RhoA/Rho-kinase is also implicated in the destabilization of nitric oxide synthase (eNOS), an important mediator of endothelial cell and smooth muscle cell function [12]. Ras, on the other hand, has been determined to regulate cell proliferation and survival

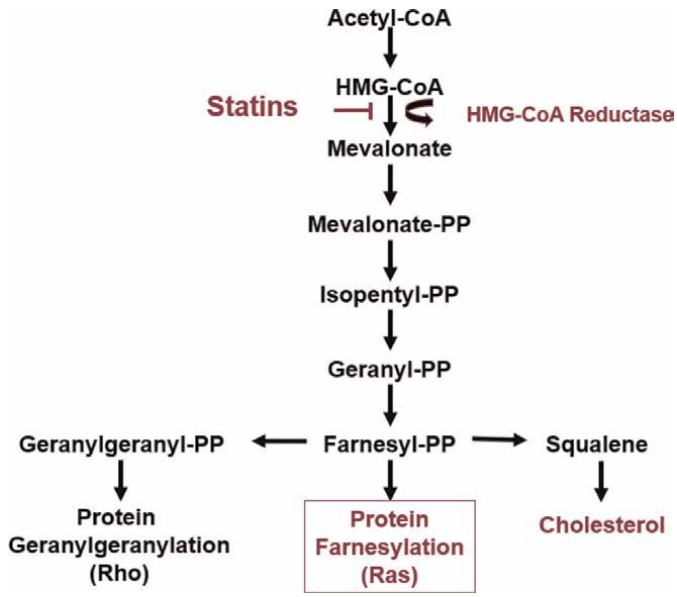

Figure 1.
Mevalonate/cholesterol synthesis pathway [8].

through the activation of effector pathways, such as Raf and mitogen activated protein kinase (MAPK) [15]. MAPK has been implicated in VSMC proliferation and migration, which is relevant to the development of PAD.

This chapter will focus on the cholesterol independent effects of statins on PAD. The prospective randomized control trials, prospective observational and retrospective studies that examine the pleiotropic effects of statins on PAD will be reviewed. Then the cellular effects of statins on atherosclerosis, intimal hyperplasia (IH), re-endothelialization, angiogenesis and arteriogenesis through cholesterol independent pathways will be presented.

2. Clinical studies

Since the late 1990s, clinical studies have shown statins (independent of lowering cholesterol) are associated with reduced major adverse cardiovascular events (MACE), having anti-inflammatory properties, improved PAD symptoms and having the potential to increase circulatory endothelial progenitor cells (EPCs). One of the first major prospective randomized clinical trials was the Heart Protection Study in 2002 (**Table 1**) [16]. This study randomized 20,536 adults in the United Kingdom with coronary artery disease (CAD), other occlusive arterial disease or diabetes to receive 40 mg simvastatin or matching placebo daily. They assessed mortality and fatal or nonfatal vascular events over a 5 year period. Irrespective of their cholesterol levels, simvastatin significantly reduced all-cause mortality, including cardiovascular and stroke related events, and nonfatal myocardial infarction (MI) or stroke. Another large prospective randomized controlled trial in 2008, the JUPITER (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin) Trial (**Table 1**), randomized 17,802 healthy men and women without hyperlipidemia to receive 20 mg rosuvastatin daily or placebo [19]. They found rosuvastatin also significantly reduced MACE. Other prospective observational studies in the early 2000s found similar results [27–29]. In 2016, Ramos et al. retrospectively assessed 5480 statin users and non-statin users aged 35–85 with PAD (Ankle-Brachial Index (ABI) <0.90), but without history of cardiovascular disease [30]. This study found statin therapy in patients with PAD was associated with reduced MI, cardiac revascularization, ischemic stroke and all-cause mortality.

To clinically measure statins effect on systemic inflammation, studies have utilized plasma concentrations of C-reactive protein (CRP) as a marker. Men and women with elevated CRP levels are known to be at increased risk of future cardiovascular events [31]. Multiple prospective randomized controlled trials have shown statins decrease plasma CRP levels at 1 month, 6 months, 1 year, 2 years, and up to 5 years (**Table 1**) [17–20]. Particularly, in patients with PAD, Bleda et al. demonstrated in sixty patients that 40 mg atorvastatin daily significantly decreased CRP levels at 1 year [20].

Studies have also indicated that statins improve leg function in patients with PAD. In 1998, the prospective randomized control trial, Scandinavian Simvastatin Survival Study, found that simvastatin (20-40 mg per day for 5 years) significantly reduced the incidence of new intermittent claudication by 38% as well as the incidence of carotid and femoral bruits (**Table 1**) [21]. In 2003, Aronow et al. showed simvastatin taken for 6 months and 1 year increased exercise treadmill time from baseline (**Table 1**) [22]. A prospective observational study performed with 392 men and women (age >55) with ABI <0.90 and 249 men and women (age >55) with an ABI between 0.90 and 1.50 showed statin users had better 6 minute walking distances and 4 meter

Reduction of Major Adverse Cardiovascular Events				
Study Name/ Author	**Selection Criteria**	**Groups**	**Primary Outcomes**	**Results**
Heart Protection Study [16]	• n = 20,536 • 40–0 years • CAD, other occlusive arterial disease or DM	**Treatment:** 40 mg simvastatin daily for 5 years **Control:** Placebo	Mortality, fatal or nonfatal vascular events	Statin ↓ all-cause mortality, coronary death rate, other vascular death rate, nonfatal MI, nonfatal or fatal stroke, and coronary or noncoronary revascularization. These results applied to all patients - LDL below 116 mg/dl or total cholesterol below 193 mg/dl.
Anti-Inflammatory Effects				
CARE trial (1999) [17]	• n = 472 • 21–75 years • acute MI 3 to 20 months before randomization	**Treatment:** 40 mg pravastatin daily for 5 years **Control:** Placebo	CRP at baseline, at 5 years. End point: death from CAD, or symptomatic nonfatal MI.	Pravastatin ↓ CRP levels at 5 years; other statins ↑ CRP levels. Changes persist after stratification by age, BMI, smoking status, blood pressure, baseline levels of total cholesterol, LDL, HDL and triglycerides.
Prince [18]	• n = 1182 with CAD • n = 1702 without CAD • > 21 years old	**Treatment:** Pravastatin 40 mg daily **Control:** Placebo	Lipid levels, CRP, other inflammatory markers at 12 and 24 weeks	Pravastatin ↓ median CRP levels (16.9%) at 12 and 24 weeks. No association between CRP and LDL levels at baseline, end of study or change over time.
Jupiter Trial [19]	• n = 17,802 • Men >50 years • Women >60 years • LDL < 130 mg/dL, CRP > 2.0 mg/L	**Treatment:** Rosuvastatin 20 mg daily **Control:** Placebo	Occurrence of MI, stroke, arterial revascularization, hospitalization for unstable angina, or death from CV causes.	Rosuvastatin ↓ LDL (50%) and CRP (37%). Rosuvastatin ↓ rates of MI, stroke, revascularization for unstable angina and for the combined end point of MI, stroke, or death from CV causes, and death from any cause. Trial stopped after 1.9 years.
Bleda [20]	• n = 60 • Fontaine grade II ischemia	**Treatment:** Atorvastatin 40 mg **Control:** Aspirin 100 mg or clopidogrel 75 mg	CRP levels, lipid levels, nitrites	Atorvastatin ↓ CRP at 1 month and 1 year. Nitrite levels (main component of oxidative stress, initial insult to endothelial dysfunction) ↓ at

Reduction of Major Adverse Cardiovascular Events				
Study Name/ Author	**Selection Criteria**	**Groups**	**Primary Outcomes**	**Results**
				1 month but not at 1 year.
Effect on PAD Symptoms				
Scandinavian Simvastatin Survival Study [21]	• n = 4444 • 35–70 years • prior MI or angina pectoris, total cholesterol 5.5–8.0 mmol/L	**Treatment:** Simvastatin 20 to 40 mg daily **Control:** Placebo	Auscultation of carotid and femoral arteries; symptoms of intermittent claudication and angina.	Follow up period of 5.4 years. Simvastatin ↓ new intermittent claudication (38%), ≥1 new bruits (30%), and new carotid bruits (48%) compared with placebo.
Aronow [22]	• n = 69 • 60–85 years • intermittent claudication, LDL >/ 125. ABI < 0.90, no prior hx of MI, angina, coronary angioplasty or coronary bypass within 6 months	**Treatment:** Simvastatin 40 mg daily **Control:** Placebo	6 months and 1 year assessed treadmill exercise (2 miles/hr., 12.5% grade).	Simvastatin ↑ treadmill exercise time until onset of claudication from baseline by 54 seconds at 6 months (vs 9 seconds in placebo) and 95 seconds at 1 year (vs −10 seconds in placebo).
Breger [23]	• n = 37 • hypercholesterolemia with stable intermittent claudication	**Treatment:** Atorvastatin 20 mg daily **Control:** Placebo	1 and 3 months assessed pain-free walking distance.	Both groups had ↑ pain free walking distance but no difference between the two groups, (at entry: 56 (53–108) m vs. 53 (53–106) m; after 3 months: 79 (53–108) m vs. 106 (66–159) m, for the treated and placebo group, respectively)
Matsumoto [24]	• n = 16 • ≥ 20 years • CLTI, unsuitable for revascularization, injected with intramuscular pitavastatin-PLGA NPs	**Treatment:** Pitavastatin-NPs	Safety, efficacy using ABI, TBI, ankle pressure, PVR, and laser doppler flow, angiography, ulcer size, degree of pain, and transcutaneous oxygen pressure.	Assessed over 26 weeks. Administration of pitavastatin-NPs was safe. Fontaine and Rutherford classification ↑ in 5 patients, ↓ in 3 patients.
Effect on EPCs				
Minami [25]	• n = 44 with stable CAD • n = 22 without CAD	**Treatment:** Atorvastatin 10 mg daily **Control:** Pravastatin 10 mg daily	Peripheral blood collected at baseline and after 12 months of therapy. Assessed EPC numbers and miR-221/222	Atorvastatin ↑ miR-221/222 in CAD group compared to non-CAD group. miR-221/222 negatively correlated with EPC number in the CAD group. After 12 months, changes in

Reduction of Major Adverse Cardiovascular Events				
Study Name/ Author	**Selection Criteria**	**Groups**	**Primary Outcomes**	**Results**
				lipid levels, atorvastatin > pravastatin. LLT with atorvastatin ↑ EPCs, ↓ miR-221/222. No change in either with pravastatin.
Vasa [26]	• n = 15 • Mean age 60 • Stable CAD; Excluded unstable angina or MI within 3 months	**Treatment:** Atorvastatin 40 mg daily **Control:** Placebo	Follow up 1 and 4 weeks. Quantity of EPC, hematopoietic precursor cells positive for CD34, CD133, CD34/kinase.	Statin treatment associated with 1.5 fold ↑ in EPC by 1 week and 3 fold ↑ by week 4. CD34/kinase insert domain increased by week 4.

Abbreviations: CAD: Coronary artery disease; DM: Diabetes mellitus; MI: Myocardial infarction; LDL: Low-density-lipoprotein cholesterol; CRP: C-reactive protein; Sig: Significantly; BMI: Body mass index; HDL: High-density lipoprotein cholesterol; HRT: Hormone replacement therapy; CK: Creatinine kinase; HTN: Hypertension; CV: Cardiovascular; ABI: Ankle-brachial index; Hx: History; M: Meters; CLTI: Chronic limb threatening ischemia; PLGA- NPs: poly (lactic-co glycolic) acid nanoparticles; EPC: Endothelial progenitor cells; LLT: Long term therapy; PAD: Peripheral arterial disease.

Table 1.
Prospective randomized controlled trials.

walking velocities after 2 years [32]. While these studies demonstrated that statins improve leg function, a randomized controlled trial by Breger et al. showed no difference in pain-free walking distance (**Table 1**); however, these patients were assessed at a shorter time interval of 1 and 3 months, suggesting that the effects of oral statins on leg symptoms may be time dependent [23]. Of note, one novel study investigated intramuscular administration of pitavastatin loaded nanoparticles to patients with chronic limb threatening ischemia unsuitable for surgery (**Table 1**) [24]. They assessed patients at 26 weeks and found administration of nanoparticles was safe and improved leg symptoms in 5 of the 16 patients.

Although there are limited clinical studies on the effects of statins on EPCs, two prospective randomized clinical trials have examined patients with stable CAD and patients without CAD (**Table 1**) [25, 26]. These studies found atorvastatin increased peripheral circulatory quantity of EPCs in patients with CAD. Suggesting statins may influence repair after ischemic injury secondary to their contribution in mobilizing EPCs.

Clinically, statins reduce cholesterol levels, and in addition through their pleiotropic effects affect the following: reduce MACE, have anti-inflammatory properties independent of lowering cholesterol, improve PAD symptoms and have the potential to increase circulatory EPCs. Although American and European guidelines recommend statins as the first line lipid lowering agent for patients with a 10-year atherosclerotic cardiovascular disease (ASCVD) risk greater than 7.5%, there remains no specific guideline that determines whether high intensity (LDL reduction >50%), moderate intensity (LDL reduction 30–50%) or low intensity (LDL reduction <30%) statin is preferred for PAD specific disease [1, 33–35]. At present, American Heart Association guidelines recommend using estimated 10 year ASCVD risk to help guide

statin intensity [35]. Recent studies have investigated whether statin intensity affects outcomes in patients with PAD. A meta-analysis by Sagris et al. found 39 studies with 275,670 patients with PAD, of which 49% were on statins [36]. Of this group, high intensity statins were associated with a 36% reduction of all-cause mortality in comparison to low intensity statins. In this study, statins were overall associated with reduction in all-cause mortality, cardiovascular mortality, MACE, risk for amputation, or loss of arterial patency. Other studies have shown similar benefits of high intensity statin therapy in PAD [37, 38]. Unfortunately, negative off-target effects, including serious muscle-related symptoms (myalgia, myositis, rhabdomyolysis), cognitive decline, hepatoxicity, new-onset diabetes, and peripheral neuropathy preclude the use of statins in some patients [39]. The population of patients intolerant of statin use is approximately 9.1% [40].

To harness the pleiotropic effects of statins while minimizing negative off-target effects, recent studies have focused on developing targeted statin therapies. These experimental drug delivery systems include statin-loaded hyaluronic acid tagged polysialic acid-polycaprolactone micelles, intramuscular or peri-sciatic injection of poly (lactic-co-glycolic acid) (PLGA) nanoparticles, and nanofiber eluting stents [24, 41–45]. We are currently investigating the effects of intraluminal administration of statin loaded chitosan-/PLGA nanoparticles on intimal hyperplasia in a rat carotid artery balloon injury model. Our goal is to optimize a delivery system that will facilitate local delivery of statins to vascular cells [46]. Preliminary data suggest that chitosan encapsulated nanoparticles are readily taken up by VSMCs (**Figure 2**). In the rest of this chapter, we will review the cellular effects of statins and incorporate novel drug delivery models that have been used to date.

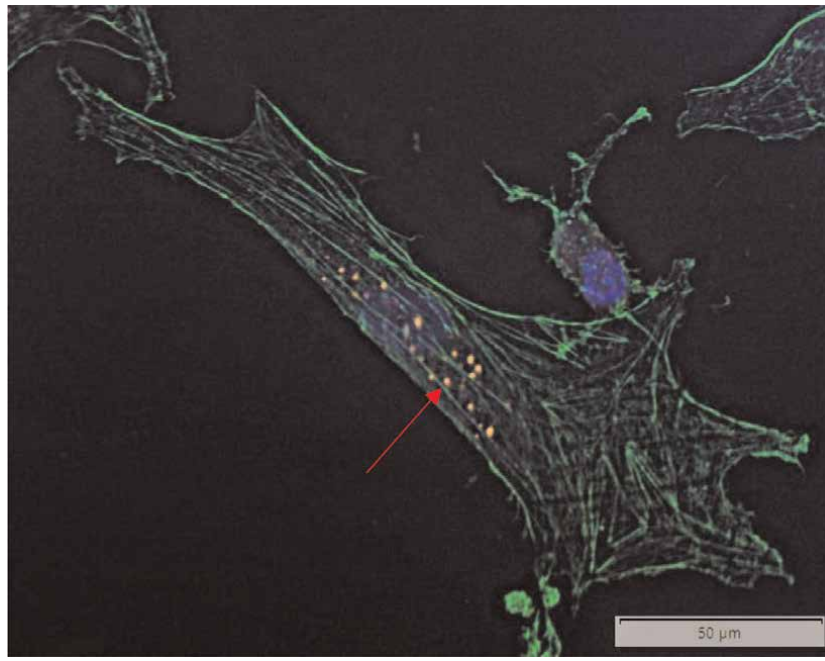

Figure 2.
Chitosan-encapsulated PLGA nanoparticles (red, Alexa Fluor 488) readily taken up within VSMCs (green, Phalloidin).

3. Cellular effects of statin

3.1 Atherogenesis

PAD can lead to acute limb ischemia as a result of embolic or thrombotic disease secondary to underlying atherosclerosis. In order to understand the pleiotropic effects of statins, to understand the pathophysiology of atherosclerosis is paramount. Atherosclerosis is a chronic inflammatory, fibroproliferative disease process that is a result of prolonged endothelial damage [47–49]. A multitude of risk factors such as hyperlipidemia, hypertension, smoking, hemodynamic factors, immune reactions, or genetic factors can lead to endothelial damage. With endothelial damage comes endothelial dysfunction, which causes a leaky and defective endothelial lining, permitting plasma molecules and lipoproteins to extravasate into the subendothelial space [47]. When low-density lipoprotein (LDL) is retained in this space, it becomes oxidized. Oxidized-LDL is cytotoxic, pro-inflammatory, chemotactic and proatherogenic [47]. The milieu created causes the endothelial cells to express adhesion molecules that help recruit monocytes and T lymphocytes. Chemoattractants then stimulate the inflammatory cells to migrate into the subendothelial space where monocytes differentiate into macrophages and internalize oxidized LDL. Internalized oxidized LDL form foam cells, or lipid-loaded macrophages, a marker of early and late atherosclerosis [47]. Foam cells that undergo apoptosis and necrosis contribute to the formation of a soft and destabilizing lipid-rich core within the atherosclerotic plaque. As the disease process progresses, VSMCs change from a contractile to proliferative and secretory phenotype [48]. This induces VSMCs to migrate from the media to the intima where they proliferate and deposit a collagen-rich extracellular matrix (ECM), forming a thick fibrous cap to the lipid-filled lesion. As the lesion grows, perfusion by the vaso vasorum becomes limited, creating a hypoxic environment that can cause VSMC death and thinning of the fibrous cap [48]. The fibrous cap is further destabilized by macrophages secreting matrix metalloproteases (MMP) and tissue factor, placing the plaque at risk for rupture. Plaque rupture then leads to the risk of thrombus formation and occlusion, resulting in limb threatening ischemia.

As described, atherosclerosis is dependent on a complex interplay between the endothelial lining, inflammatory cells, lipoproteins and VSMCs. Of which, elevated plasma cholesterol levels have shown a clear association with increased atherosclerotic disease. Statins, therefore, were a promising medication to help minimize atherosclerotic burden by lowering plasma cholesterol levels. Particularly, statins are known to decrease LDL and remove existing LDL from the circulation; however, studies have demonstrated the overall benefits of statins are greater than anticipated due to their pleiotropic effects [29]. For example, in a cholesterol independent manner, statins stabilize atherosclerotic plaque, decrease endothelial dysfunction and reduce vascular inflammation.

3.1.1 Statin effect on plaque stability

Statins contribute to plaque stability by decreasing macrophage accumulation and inhibiting MMP and tissue factor production by cholesterol independent and dependent mechanisms [9, 14]. Previously, statins were thought to stabilize plaque through their cholesterol lowering mechanism, as lowering cholesterol via dietary mechanisms decreased macrophage and proteolytic enzyme accumulation [50]. This effect of

statins was highlighted by Crisby et al's study, which demonstrated atherosclerotic plaque (collected from carotid endarterectomy) in patients taking pravastatin for 3 months contained less lipid, higher interstitial collagen, less oxidized LDL, fewer macrophages, less MMP-2 and greater tissue inhibitor of MMP-1 in comparison to patients who received no statin therapy [51]. Later studies demonstrated statins work through additional mechanisms to stabilize plaques. For example, in Feig et al's study, they demonstrated statins not only lower cholesterol to decrease macrophage accumulation, but, actively induce macrophage emigration through the chemokine receptor CCR7 [52]. Taken together, statins work through cholesterol dependent and independent mechanisms to reduce proteolytic enzyme activity, thereby reducing the risk of destabilizing the fibrous cap on atheromas.

3.1.2 Statin effect on endothelial dysfunction

One of the earliest components of atherosclerosis is endothelial dysfunction, which leads to the impaired synthesis, release and activity of endothelial-derived nitric oxide (NO) [9]. NO is a soluble gas synthesized by nitric oxide synthase (eNOS). NO is critical for vascular hemostasis due to its vasodilatory, anti-inflammatory and antioxidant properties [53]. Once endothelial dysfunction ensues, reactive oxygen species propagate its dysfunction. Statins have been documented to improve endothelial function by increasing NO production and reducing reactive oxygen species.

Statins minimize endothelial dysfunction by increasing eNOS and stabilizing eNOS mRNA, thereby increasing NO production [9, 54, 55]. Statins increase NO production through multiple mechanisms, including its interaction with RhoA, phosphatidylinositol 3-kinase (PI3k)/AKT and caveolin-1 [9, 56]. As previously discussed, RhoA downregulates eNOS and statins reduce RhoA through reducing production of mevalonate [57]. Therefore, statin mediated inhibition of the isoprenoid intermediate RhoA results in reduced inhibition of eNOS. In regards to PI3k/Akt, statins have been shown to enhance its phosphorylation, which increases Akt expression [58, 59]. Akt has been shown to enhance eNOS expression. Caveolin-1, on the other hand, is an inhibitory protein that binds eNOS, inhibiting the production of NO [56, 60]. Statins have been shown to decrease the expression of caveolin-1, thereby increasing eNOS production of NO [56]. Through these mechanisms, statins work to enhance eNOS which increases NO production and reduces endothelial dysfunction. Statins further reduce endothelial dysfunction through its antioxidant effects. Moon *et al* demonstrated that rosuvastatin administered to 99 patients who had experienced an atherosclerotic stroke resulted in a reduction of oxidative stress markers (specifically, malonidialdehyde and oxidized LDL) compared to patients without treatment [61]. The reduction of oxidative stress markers was thought to be secondary to statins ability to inhibit oxidant enzyme activity, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and myeloperoxidase, while upregulating antioxidant enzymes, catalase and paraoxonase [62]. Further studies have demonstrated statins reduce reactive oxygen species through enhanced phosphorylation of PI3k/Akt and activation of the transcription factor Nfr2 [58]. Through these pleiotropic effects, statins help decrease reactive oxygen species and therefore reduce the progression of atherosclerosis.

3.1.3 Statin effect on vascular inflammation

Chronic inflammation is a large component of the initiation and progression of atherosclerosis. Statins appear to reduce vascular inflammation by reducing leukocyte

adhesion and transendothelial migration [45, 63, 64]. Simvastatin in particular, has been shown to decrease leukocyte rolling and adhesion *in vitro,* and *in vivo* [65]. Other studies have shown that statins reduce the leukocyte-endothelial interaction by decreasing the RhoA-dependent expression of endothelial adhesion molecules including P- and E- selectins, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 [66, 67]. Statins also hinder the recruitment of inflammatory cells into the vascular intima and across inflamed vascular endothelium by attenuating the expression of monocyte chemoattractant protein-1 (MCP-1) via a mechanism that may involve Cdc42 and RalA GTPases [63, 68–70]. Taken together, statin pleiotropic effects hinder the propagation of atherosclerosis, in part, by inhibiting leukocyte transendothelial migration.

In summary, statins stabilize atherosclerotic plaques by reducing macrophage and MMP accumulation, improve endothelial function by stabilizing and increasing NO via increased eNOS production, and diminish vascular inflammation by hindering leukocyte adhesion and transendothelial migration. The pleiotropic effect of statins on atherosclerosis is largely through inhibition of mevalonate-derived products, as well as through other pathways, including PI3k/Akt.

3.2 Intimal hyperplasia

When PAD is significant enough to warrant intervention, the management strategy can be through endovascular procedures (angioplasty, stenting) or surgically (endarterectomy or bypass); however, restenosis secondary to IH remains a significant challenge that affects patency rates after intervention [71–73]. IH is triggered by endothelial damage, which can be caused by balloon inflation, stent implantation or bypass graft placement [12, 73–75]. The endothelial damage that ensues stimulates the production of proinflammatory molecules and activation of circulatory monocytes that bind and penetrate the vascular wall, perpetuating a local inflammatory response. There is also a reduction of VSMC inhibitory factors, such as heparan sulphate, NO and natriuretic peptides. Simultaneously, there is production of plasminogen activators that contribute to the degradation of the ECM and activation of MMPs [74]. These factors together stimulate VSMC transition from a contractile state to a proliferative state through MAPKs [76]. Migration and proliferation of VSMCs from the medial and adventitial layer of the arterial wall into the intimal or subendothelial space then ensues. Intimal expansion subsequently occurs, secondary to VSMC accumulation and exuberant ECM synthesis stimulated by growth factors, such as TGF-beta and platelet derived growth factor [77].

One mechanism by which statins reduce IH is through the inhibition of the small GTPase RhoA [12]. As previously discussed, RhoA interacts with Rho-kinase to promote vascular contraction, VSMC migration and reduce eNOS gene expression, steps crucial in the pathophysiology of IH [78]. Therefore, by statins inhibiting RhoA, we would anticipate decreased VSMC migration, as well as the restoration of NO production. In 2005, Yamanouchi et al. demonstrated normocholesterolemic rats supplemented with oral pravastatin 10 mg/day exhibited reduced vein graft IH with suppressed cellular proliferation and increased cellular apoptosis [79]. *In vitro,* they demonstrated human umbilical vein endothelial cells (HUVECs) treated with pravastatin inhibited Rho-kinase activity and accelerated endothelial eNOS expression; however, there was no effect on Rho-kinase in VSMCs. Other *in vivo* studies have shown oral and intraperitoneal statins (pitavastatin, simvastatin, atorvastatin) reduce IH [80–84]. Studies using novel techniques have also assessed the effect of statins

effect on IH [41, 85]. These techniques have been developed to locally deliver statins in one dose for the purposes of reducing systemic toxicity. For example, Helkin et al. assessed simvastatin loaded in hyaluronic acid tagged polysialic acid-polycaprolactone micelles delivered to rats intraluminally prior to carotid artery injury. This treatment demonstrated a 55% reduction of IH while oral simvastatin reduced IH by 25%. Other studies have incorporated statins into drug-eluting stents, with statin-eluting-stents decreasing IH in comparison to other stents [44, 86]. Particularly, Tsuki et al. developed a bioabsorbable statin incorporated polymeric nanoparticle-eluting stent [44]. They demonstrated in a pig coronary artery model, pitavastatin incorporated nanoparticle eluting stent attenuated in-stent stenosis, comparably to sirolimus-eluting stents, without delayed endothelial healing. Future studies are aimed at finding alternative means to deliver localized statins at the site of endothelial injury to minimize risks of systemic therapy.

Another cholesterol independent effect of statins is their inhibition of another small GTPase protein, Ras. Sakamoto et al. assessed the effect of fluvastatin on MAPKs, extracellular signal-regulated kinase 1 and 2 (ERK1/2) and p38MAPK phosphorylation in an organ-cultured rat tail artery [76]. ERK1/2 and p38MAPK are believed to be phosphorylated and activated by Ras, inducing the morphologic change of contractile VSMCs to the proliferative state [87]. They demonstrated that fluvastatin significantly decreased ERK1/2 and p38MAPK and restored VSMC contractility, suggesting that statins may inhibit proliferation via inhibition of MAPK phosphorylation. In 2020, Chu et al. evaluated the effect of atorvastatin on the phosphorylation of p38 MAPKs [81]. They found rats undergoing vein graft bypass had reduced IH and significantly decreased p38 MAPK phosphorylation ($p < 0.05$) when treated with oral atorvastatin. These studies indicate that the pleiotropic effects of statins hinder the progression of IH not only via endothelial cell dependent mechanisms but also via inhibition of VSMC migration.

In summary, statins decrease IH at least through two different pathways that are activated by RhoA and Ras. By inhibiting RhoA, statins inhibit Rho-kinase which decreases VSMC migration and NO production. By inhibiting Ras, statins inhibit the p38MAPK pathway, which decrease VSMC migration and proliferation.

3.3 Re-endothelialization

The leading pathophysiology of atherosclerosis and IH is endothelial damage. To accommodate for this, endothelial cells are continuously attempting to repair themselves by proliferating resident endothelial cells and circulating EPCs [88]. This concept of repair is termed re-endothelialization. Statins accelerate re-endothelialization by mobilizing, differentiating, and improving survival of EPCs [89–91]. A study by *Walter et al* demonstrated simvastatin administered to Sprague–Dawley rats accelerated re-endothelialization after carotid artery balloon injury and resulted in a dose dependent reduction of neointimal thickening [92]. In regards to re-endothelialization, simvastatin was shown to increase the rat's circulating EPCs and induced adhesiveness at the site of endothelial injury by upregulating the integrin subunits alpha5, alpha(v), beta1 and beta5. By upregulating these integrin subunits, statins not only mobilize EPCs, but target them to the site of endothelial injury. Further studies demonstrated statins increase circulating EPCs through the phosphorylation of Akt, activating the PI3k/Akt signaling pathway [89]. These findings establish yet another pleiotropic mechanism by which statins specifically pre-empt vascular wall pathologies.

3.4 Angiogenesis and Arteriogenesis

Angiogenesis, in the adult, is the formation of new capillaries. It features sprouting of new endothelial cells from preexisting capillaries under the influence of angiogenic factors generated by a hypoxic environment [93, 94]. Arteriogenesis describes the remodeling of preexisting collateral arterioles. Typically, the collateral arterioles are high resistance and do not offer much blood flow to distal capillary beds. With proximal arterial occlusion, hemodynamic changes provoke arteriole remodeling, which encompasses proliferation of vascular cells and turnover of the vascular matrix [93, 94]. The complex interplay that ensues between vascular cells, adhesion molecules, chemokines and monocytes results in collateral arterioles with increased diameter and wall thickness, providing a natural bypass; however, this adaptation is not equivalent to direct arterial perfusion.

In patients with progressive PAD, endovascular or surgical revascularization is the preferred therapeutic strategy. However, not all patients are candidates due to the severity of disease or due to risk of intervention secondary to a patient's severe comorbidities. In this population, patients rely on angiogenesis and arteriogenesis to promote perfusion to ischemic tissue. Unfortunately, endogenous angiogenesis and arteriogenesis are not enough to restore blood flow in the setting of critical limb ischemia. Therefore, a growing body of literature has investigated medical interventions including medical revascularization with the goal of increasing blood vessel growth and improving perfusion to ischemic extremities [93]. One of these medical interventions includes administration of statins.

3.4.1 Statin effect on angiogenesis

Statins have a dose-dependent biphasic effect on angiogenesis, with lower-doses acting in a pro-angiogenic manner and higher doses acting in an anti-angiogenic, pro-apoptotic manner [95–99]. The pro-angiogenic effects of statins appear to act through the intracellular signaling pathway, PI3 kinase/Akt (**Table 2**) [59, 101–104]. One effect of enhancing the PI3 kinase/AKT signaling pathway is increasing capillary density. One study used both in vitro and in vivo models to demonstrate that statins enhance phosphorylation of Akt, increasing its substrate eNOS, which inhibited endothelial cell apoptosis and accelerated formation of endothelial cell tubules in a matrigel assay [59]. *In vivo,* statins promoted angiogenesis and blood flow recovery in a rabbit hindlimb ischemia model. One study suggested that the statin effect may be mediated by Notch1 signaling, a downstream effector of PI3Kinase/Akt (**Table 2**) [104]. Statins also influence angiogenesis through their effect on EPCs. EPCs have been demonstrated to be involved in neovascularization, thereby constituting post-natal vasculogenesis [95]. Statins have been shown to promote the survival, migration and differentiation of adult EPCs through Akt dependent mechanisms [97]. For example, *in vitro* low dose fluvastatin was shown to activate the Akt/eNOS pathway resulting in increased EPC migration and proliferation [101]. This same study showed *in vivo* enhanced incorporation of EPCs in ischemic tissue with increased capillary density.

While lower doses of statins promote angiogenesis, high doses of statins have been shown to be anti-angiogenic and pro-apoptotic (**Table 2**). One mechanism by which high dose statins may decrease angiogenesis is through their inhibition of RhoA [97]. Weis *et al.* demonstrated *in vitro* that high doses of statins increased endothelial cell apoptosis, inhibited endothelial cell proliferation, migration, differentiation and

Statin	Dose	Cell Type/Model	Biologic Effect	Reference
Simvastatin/ Rosuvastatin	1 μM and 10 μM	HUVECS/Ex vivo mouse aortic ring	***In vitro:*** Statins↑ HUVEC tube formation at low dose. ***Ex vivo:*** Statins abolished new vessel formation, ↑ VE-cadherin and cell–cell adhesion.	Khaidakov 2009 [99]
Fluvastatin + TSP-5	1 μM Fluvastatin; 20 μg/ml TSP-5	TSP-5 treated human aortic ECs	Statin reverses antiangiogenic effects of TSP-1 and TSP-2; ↓ proapoptotic genes and apoptosis; ↑ proangiogenic genes and angiogenesis.	Muqri 2020 [98]
Cervistatin/ Atorvastatin	***In vitro:*** [Low]: 0.005–0.01 μM [High]: 0.05–1 μM ***In vivo:*** [Low]: 0.5 mg/kg/day [High]: 2.5 mg/kg/ day	Human adult dermal microvascular ECs / WT and hypercholesterolemic C57BL/6 J mice 24 weeks	Statin ↑ EC proliferation, migration and differentiation at [low], ↓ at [high]. Antiangiogenic effects associated with ↓ EC release of VEGF, ↑ EC apoptosis. Effects reversed by GGPP. ***In vivo*:** Statin ↑ angiogenesis at [low] dose, ↓ at [high] dose in WT and hypercholesterolemic mice.	Weis 2002 [100]
Fluvastatin + stromal cell derived factor-1	***In vitro:*** Fluvastatin at 10 nM, 100 nM and 1000 nM; SDF-1100 ng/mL. ***In vivo:*** 5 mg/kg statin	Rabbit EPCs / C57BL/ 6 J mouse hindlimb ischemia model	***In vitro*:** 100 nM fluvastatin or 100 ng/ml SDF-1 ↑ EPC proliferation and migration, ↓ EPC apoptosis, ↑ MMP-2, MMP-9, Akt phosphorylation and NO. ***In vivo:*** ↑ reperfusion ratio, cell proliferation, EPC incorporation, capillary density, ↓ apoptosis.	Shao 2008 [101]
Rosuvastatin	[Low]: 0.1 mg/kg [High]: 5 mg/kg	Mouse EPCs / Mouse hindlimb ischemia model	***In vitro:*** Rosuvastatin ↑ p-Akt/p-eNOS levels. ***In vivo:*** Rosuvastatin ↑ capillary density and blood flow recovery. eNOS deficient mice, increase blunted.	Zhou 2013 [102]
Simvastatin/ Pravastatin	***In vitro:*** 1.0 μM ***In vivo:*** 0.1 mg/kg	HUVECs / Rabbit hindlimb ischemia model	***In vitro:*** Statin ↑ phosphorylation of Akt, inducing phosphorylation of eNOS, causing NO production; ↑ tubles in matrigel, ↓ apoptosis. ***In vivo:*** statins ↑ capillary formation.	Kureishi 2000 [59]
Lovastatin	1–10 μM	HUVECs	Lovastatin ↑ actin-binding protein transgrelin 2 causing ↓ MLC phosphorylation. MLC phosphorylation inhibition reversed with transgelin 2 knockdown. Rho inactivation associated with	Xiao 2012 [103]

Statin	Dose	Cell Type/Model	Biologic Effect	Reference
			↑ transgrelin causing ↓ HUVECs migration and tube formation.	
Pitavastatin	***In vitro:*** 100 nM ***In vivo:*** 1 and 3 mg/kg/day	HUVECs / C57BL/6 J WT mice; Notch1 heterozygous-deficient mice; hindlimb ischemia model	***In vitro*:** Statin activated endothelial y-secretase and Notch1 downstream of PI3K/akt (100 nmol, ↓ at 1microM) and ↑ ephrin B2 downstream (promoter of arteriogenesis). ↑ proangiogenic activity of ECs, ameliorated by Notch 1 inhibition. ***In vivo*:** Statin (3 mg/kg) ↑ blood flow recovery, capillary density, arterioles in WT mice but not in Notch1 deficient mice.	Kikuchi 2011 [104]

Abbreviations: EC: endothelial cell; EPC: Endothelial progenitor cell; HUVEC: human umbilical vein endothelial cell; TSP-5: Thrombospondin-5; GGPP: geranylgeranyl pyrophosphate; WT: Wild-type; MLC: Myosin light chain; VEGF: vascular endothelial growth factor; eNOS: nitric oxide synthase; NO: nitric oxide.

Table 2.
Angiogenic effects of statins.

decreased endothelial cell release of VEGF (**Table 2**) [100]. *In vivo*, mice given high dose statin had an angiostatic response after hindlimb ischemia. The *in vitro* anti-angiogenic effects were reversed with the administration of GGPP. Another mechanism by which statins may inhibit angiogenesis is through their increase of cell cycle inhibitors and downregulation of angiogenesis related genes (PAI-1, vitronectin, HoxD3, and Notch4) [105]. Interestingly, studies have indicated high doses of statin continue to stabilize eNOS mRNA, further demonstrating the duality of statins [106].

The biphasic angiogenic effect of statins is complex and may be due to the differing affinity for GGPP/RhoA and FPP/Ras inhibition. Zahedipour et al. have suggested that at low doses, statins preferentially inhibit cholesterol synthesis, which leaves products of GGPP/RhoA and FPP/Ras uninhibited [97]. By contrast, high dose statins significantly reduce all byproducts of the mevalonate pathway. The effect is reduction in cell proliferation and migration, consistent with the angiostatic environment high dose statins produce. Future studies will be needed to determine the optimal dosage and vehicle for statins to be used as a novel medical revascularization technique.

3.4.2 Statin effect on Arteriogenesis

In the past decade, studies have demonstrated that statins induce arteriogenesis. In particular, pitavastatin has been shown to increase angiogenesis and arteriogenesis in a murine hindlimb ischemia model [104]. Other studies have used novel drug carriers (PLGA nanoparticles) to administer pitavastatin into ischemic muscle of mice, rabbit and cynomolgus monkey [42, 43]. These studies demonstrated that intramuscular injection of polymeric nanoparticles delivering 0.5 mg/kg pitavastatin induced arteriogenesis and ameliorated exercise-induced ischemia. *In vitro* studies suggest that the effect of statins on arteriogenesis appears to be through Notch1 and Akt signaling. In the study by *Kikuchi et al*, they demonstrated pitavastatin activated Notch1 in

HUVECs, which was inhibited by knockdown of Akt [104]. *In vivo,* they demonstrated that pitavastatin administered to wild-type mice undergoing hindlimb ischemia increased endothelial ephrin B2 (a downstream target of Notch1) and induced arteriogenesis. This effect was not apparent in Notch1 mutant mice, suggesting Notch1 facilitates the arteriogenic effects of statins. Similarly, *Zacharek et al* demonstrated rats subjected to middle cerebral artery occlusions treated with simvastatin demonstrated increased arteriogenesis [107]. Rats with increased arteriogenesis also demonstrated upregulation of Notch1 and its effector, notch intracellular domain. This effect was mitigated by inhibition of Notch signaling. These studies demonstrate statins effect of increased arteriogenesis is through activation of Notch1 and enhanced Akt signaling.

4. Conclusions

The prevalence of PAD is growing globally secondary to our aging population. The management of PAD is multi-disciplinary and begins with prompt risk factor management and initiation of medical therapies. Of these medical therapies, statins have been shown to be a great benefit given their cholesterol lowering abilities and pleiotropic effects. As described, cholesterol independent mechanisms of statins include stabilizing atherogenic plaques, reducing IH, increasing reendothelialization via mobilizing of EPCs, and promoting angiogenesis in a dose-dependent manner. The pleiotropic effects of statins are largely mediated through inhibition of mevalonate-derived products, such as RhoA and Ras GTPases, as well as through phosphorylation and activation of the PI3k/Akt pathway. The effects of statins on these pathways include increased NO production and endothelial stabilization, reduced vascular inflammation, reduced VSMC proliferation, increased EPC mobilization, decreased intimal hyperplasia, variable effects on angiogenesis and increased arteriogenesis. Future studies aim to develop techniques that will safely provide local delivery of statins to the area of PAD, in hopes of minimizing systemic toxicities and providing medical revascularization.

Abbreviations

ABI	ankle-brachial Index
ASCVD	atherosclerotic cardiovascular disease
CAD	coronary artery disease
CRP	c-reactive protein
ECM	extracellular matrix
eNOS	nitric oxide synthase
EPCs	endothelial progenitor cells
ERK1/2	extracellular signal-reglated kinase 1 and 2
FPP	farneyslpyrophosphate
GGPP	geranylgeranylpyrophosphate
GTP	guanosine triphosphate
HUVECs	human umbilical vein endothelial cells
IH	intimal hyperplasia
LDL	low-density lipoprotein
MACE	major adverse cardiovascular events

MAPK	mitogen activated protein kinase
MCP-1	monocyte chemoattractant protein-1
MI	myocardial infarction
MLC	myosin light chain
MMP	matrix metalloproteases
MYPT1	myosin phosphatase, target subunit 1
NAD[P]H	nicotinamide adenine dinucleotide phosphate
NO	nitric oxide
p38MAPK	p38 mitogen activated protein kinase
PAD	peripheral arterial disease
PDGF	platelet derived growth factor
PI3K	phosphatidylinostiol 3-kinase
VSMCs	vascular smooth muscle cells
PLGA	poly (lactic-co-glycolic acid)

Conflict of interest

The authors declare no conflict of interest.

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Chapter 3

Statins Effects on Venous Wall in Patients with Chronic Venous Disease

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Abstract

The anti-atherogenic, anti-inflammatory, and vasomotor effects of statins on the arteries are well known, but there are no significant literature data about statins treatment effects on veins. This study describes the potential morphological changes that may occur in the venous wall in the case of the patients with chronic venous disease (CVD) which associates atorvastatin treatment for at least two years. The patients were clinically evaluated, and at the same time, a microscopic morphological analysis was performed on surgically removed veins fragments. The obtained results prove an improvement in the CVD prognosis, as well as a better postoperative evolution in these patients, and suggest an improvement in the blood flow in the vasa vasorum from the venous adventitia, as well as a potential phlebotonic and phlebotrophic effect of statins.

Keywords: statins, veins, chronic venous disease, blood flow improvement, phlebotonic effect

1. Introduction

Chronic venous disease (CVD) is a prevalent condition with global spread, affecting about a quarter of the adult population, significantly influencing the quality of life of these patients. The prevalence of CVD is highest in Western countries. More recent epidemiological studies of venous diseases in which the CEAP classification was used show a prevalence of 60–70% CEAP clinical class C0 and C1, about 25% for C2 and C3, and up to 5% for C4 to C6 with skin changes or venous ulcers. A recently published comprehensive systematic review on global epidemiology of CVD including >300,000 adults showed that the pooled prevalence of C2 disease is increasing. C2 disease had a progression rate of 22% developing a venous leg ulcer in six years. The incidence of varicose veins is approximately 2% per year [1–3]. At the same time, the incidence of cardio-metabolic diseases is worldwide increasing because of an increase in the standard of living [4], and in many cases, these diseases are associated with varicose veins of the lower limbs due to common risk factors such as obesity, sedentary lifestyle or inadequate nutrition [5].

Dyslipidemia is defined by the presence of nonoptimal levels of blood lipids, and it is a common risk factor for cardiovascular diseases. In clinical practice guidelines,

dyslipidemia is mostly defined by elevated total cholesterol and/or low-density lipoprotein cholesterol, but the definition is also often extended to include nonoptimal levels of high-density lipoprotein cholesterol, triglyceride, apolipoprotein B, and apolipoprotein A1. Because dyslipidemia is one the most important modifiable risk factors for cardiovascular diseases [6], treatment is frequently indicated as primary prevention, even at an early age, statins being currently the most commonly prescribed drug worldwide for lowering serum cholesterol [7].

The beneficial effects of statins on clinical events may involve nonlipid mechanisms that modify endothelial function, smooth muscle cells, and monocyte–macrophage: vasomotor function, inflammatory responses, and plaque stability. Augmented bioactivity of nitric oxide by statins therapy either indirectly by its effect on lipoprotein levels and protection of LDL from oxidation, or directly by effects on nitric oxide synthesis and release, might account for enhancement of endothelium-dependent vasodilation. Recent experimental and animal studies have demonstrated that statins dose-dependently decrease smooth muscle cells migration and proliferation, independently of their ability to reduce plasma cholesterol. Moreover, statins are able to reduce the in vitro cholesterol accumulation in macrophages and expression of matrix metalloproteinase, resulting in plaque stability. These effects of statins were completely prevented by the addition of mevalonate and partially by all-trans farnesol and all-trans geranylgeraniol, confirming the specific role of isoprenoid metabolites, probably through prenylated proteins, in regulating these cellular events. Statins have been shown to prevent the activation of monocytes into macrophages, inhibit the production of pro-inflammatory cytokines, C-reactive protein, and cellular adhesion molecules. Statins decrease the adhesion of monocyte to endothelial cells. Accordingly, statins exert their cardiovascular benefits through a direct antiatherogenic properties in the arterial wall, beyond their effects on plasma lipids [8].

The anti-atherogenic, anti-inflammatory, and vasomotor functions of statins on the arterial wall are well known [8, 9], and despite the fact that the pleiotropic effects of statins have been documented for over 20 years [10], only a few studies debate the advantages of statins in healing venous leg ulcers, and there are no studies documenting how statins act on the venous wall morphology, and particularly in patients diagnosed with CVD.

This paper represents a sequel of a previous study [11] regarding the impact of statin medication in the case of the phlebological patient and the morphological changes that may occur in the venous wall in this case, debating additionally the clinical evolutions of those patients and the clinical implications of these observations.

2. Methodology

2.1 Initial study data

A series of morpho-anatomical parameters for a study group and a control group, which analyzed patients with CVD, were compared in the initial study. Those parameters included age (years), BMI (kg/m^2), lumen (thrombosed/free), maximum venous diameter (cm)—it took into account the maximum diameter at the level of the vein of the largest caliber from the surgically excluded specimens, for each patient—venous wall thickness (cm); thickening of TI; thickening of TM; disorganized muscle bundles in TM; fragmentation of elastic fibers in TM; collagen increased in TA; absence of vasa vasorum in TA. This research study was a retrospective one.

A number of inclusion criteria were common for both groups, as follows: CVD, C2–C3 CEAP stage; an average period of 60 +/− 4 months from the first presentation to a physician for venous disorders symptoms, for each patient; venous reflux affecting superficial venous system, objectivated by Doppler ultrasound; no previous endovenous treatment (thermal or nonthermal ablation, foam, etc.); outpatient admission for CVD surgical treatment; serum cholesterol values below 180 mg/dl; signing an informed consent for study participation.

The specific inclusion criterion for the SG was chronic atorvastatin treatment for a period of at least two years' time (range: 29 +/− 2 months), dosage: 10 mg/day.

According to those criteria, 50 patients were included in the study group, and 52 patients were included in the control group. Regarding the therapeutic indication for atorvastatin treatment among the patients in the study group, 39 patients had high serum cholesterol values at the time of starting the treatment, and 11 patients were using this treatment as primary prevention.

Before surgery, all the patients were examined by duplex ultrasound. All those patients were subsequently operated for venous insufficiency. In accordance with the dimensions and topographic position of the veins that had to be surgically treated, different opening procedures (phlebectomies, classic stripping, or cryostripping) were performed. According to the preoperative mapping of the superficial venous network of the lower limb, only the veins that showed reflux during the ultrasound examination were surgically excluded.

A total of 215 fragments of varicose veins (great saphenous veins, small saphenous veins, or/and their venous tributaries) were collected from the SG, and 179 fragments of varicose veins were collected from the CG. The specimens were collected in fixative solution (10% buffered formalin) and then were analyzed by the Pathology Laboratory. The collected fragments were analyzed macroscopically and microscopically. The macroscopic analysis evaluated the maximum diameter, the thickness of the venous wall, and the aspect of the lumen (free/thrombosed).

For the microscopical analysis, all the venous fragments were prepared on microscope slides. In this regard, several cross-sections were cut from the areas of maximum wall thickness. Any existing thrombus was included. The processing of the parts was performed manually by dehydration in alcoholic solutions, clearing, paraffining, and performing paraffin inclusion, and sections with a thickness of 4 micrometers were made using the semi-automatic rotary microtome Medite M530. For each case, two successive sections were obtained, these being colored in the usual Hematoxylin–Eosin stain, and the Masson trichrome (with aniline blue) stain to highlight fibrosis. The coloring was performed using Bio Optica reagents, by using an automatic Leica Autostainer XL stainer, following the standard recommended protocols. Microscopically, the evaluation was a qualitative one, tracking the changes at vascular components level: thickening of the intima, thickening of the media, with the eventual disorganization of muscle bundles and/or fragmentation of elastic fibers, respectively, increased collagen quantity and the presence/absence of vasa vasorum in the adventitia. A team of two pathologists analyzed the sections displayed on the slides, using a Zeiss Axioskop 2 Plus microscope. The results represent the common conclusion of both doctors.

The aim of this study was to identify and compare the potential morphological changes that may occur in the venous wall in CVD patients due to the atorvastatin treatment.

2.2 Follow-up and clinical implications

This study represents the continuation of the previous one and followed the clinical implications of the effects of statin treatment in the patient with CVD how that may influence disease progression and postsurgical evolution for both groups of patients, being followed by a series of parameters, early and one year postoperatively. The following data were analyzed: hospitalization period, complications (bruising <2 cm surface, bruising = 2–10 cm surface, hematoma), the postoperative outcomes and the recurrences.

2.3 Statistical analysis

Data obtained from the control and study groups were statistically analyzed using Microsoft Excel. The chi-square test ($\chi 2$ test) was used, and for greater accuracy, for the small sample size, we used Fisher's exact test. Student's t distribution test was also used for small study groups with continuous distribution of values. In order to have independent results between the values obtained, the Pearson correlation coefficient was applied. We specify that the resulting p-value below <0.05 was considered statistically significant.

2.4 Ethical approval

This research was conducted at the Timișoara Emergency County Hospital, in the Phlebology Department, with the consent of the Ethics Commission (REC number: HR.233/04.03.2021, REC number: NR. 332/14.10.2022) and in accordance with the rules of the Helsinki Declaration.

3. Results

For the study group, the age range varied between 44 and 73 years (mean age–56.32). For the control group, the age range varied between 43 and 75 years (mean age–55.08 years). Regarding this parameter, the groups were statistically equivalent.

Body-mass-index analyses revealed normal distribution in the groups. Student's t test was used for the statistical comparison between groups, but the result did not reveal significant differences ($p = 0.95$). From the descriptive point of view, the variation range of BMI was 19.7–37.9 kg/m^2 (average 28.25 +/−4.36 kg/m^2; median 28.25 kg/m^2) for the study group, while the BMI range of the control group was within 20.5–38.2 kg/m^2 (average 28.3 +/− 4.02 kg/m^2; median 28.4 kg/m^2).

Intravenous thrombi were not identified in many cases, the most frequent being observed free venous lumen (study group – 45 patients: free lumen, 5 patients: the lumen was occupied by thrombus; control group –43 patients: free lumen, 9 patients: the lumen was occupied by thrombus). There were not observed statistically significant differences were between the groups ($p = 0.28$).

Maximum venous diameter analyses revealed in the study group values which ranged between 0.2–1.3 (mean 0.45 +/− 0.303; median 0.45), while in the control group the values ranged between 0.2 and 1.6 (0.781 +/− 0.29; median 0.7). From a statistical point of view, highly significant differences are highlighted for this parameter when comparing the two groups ($p = 0.00078$). In the case of the SG, a functional impairment is observed predominantly in the small-caliber veins (venous tributaries

and collateral veins), while in the CG the major trunks (great saphenous veins, small saphenous veins) were affected in most of the patients.

For the venous wall thickness parameter, no statistical differences between the analyzed groups were noted. Descriptively, values between 0.1 and 0.5 centimeters were found in SG (average of 0.21 +/− 0.1; median 0.2), while in CG, the values ranged between 0.1 and 0.6 centimeters (average 0.2 +/− 0.14; median 0.2).

A statistically significant difference between groups was observed regarding thickening of the venous TI ($p = 0.04$). Venous TI thickening was identified in 20 patients from SG, and in 31 patients from CG.

Analyses of venous TM thickening, with the four cases found (collagen; collagen + muscle fibers; muscle fibers; without thickening) revealed statistically significant differences ($p = 0.0079$) between groups. Results regarding TI and TM thickening are presented in **Table 1**.

The presence of disorganized muscle bundles in the TM did not revealed statistically significant differences between groups ($p = 0.11$). Also, the analysis of the fragmentation of elastic fibers from TM showed insignificant results, also ($p = 0.57$). A weak correlation (0.66) between the disorganized muscle bundles and the fragmentation of the elastic fibers in the TM was observed in the study group, fact that was noted in the control group (0.57), too. This observation suggests that the correlation of these parameters is not a consequence of the atorvastatin treatment.

No statistically significant differences between the two groups ($p = 0.92$) regarding the presence of increased collagen in TA was observed.

In this study, the absence of vasorum vessels in TA was an interesting parameter. Despite the fact that it provided significant "p" values in the $\chi 2$ test, the results were slightly above the minimum value of 0.05 in the Fisher test. For that reason, it could be considered a questionable result.

The morphology of the analyzed specimens is presented in **Figure 1** for SG and **Figure 2** for CG.

Analyzing the correlations identified with respect to the studied parameters, the closest correlation was found between wall thickness and increased collagen in TA (0.83), a correlation that was not observed in CG. We can, therefore, consider this fact to be related to the atorvastatin treatment. To a slightly lower degree (0.61),

Thickening of tunica intima (TI)	**study group**	**control group**
yes	20	31
no	30	21
χ^2Test p = 0.047628		
Thickening of tunica media (TM)	**study group**	**control group**
yes, collagen	40	26
yes, collagen + muscle fibers	5	11
yes, muscle fibers	5	11
no	0	4
Fisher's Exact Test p = 0.0079		

Table 1.
Analysis of tunica intima (TI) and tunica media (TM) thickening for both groups.

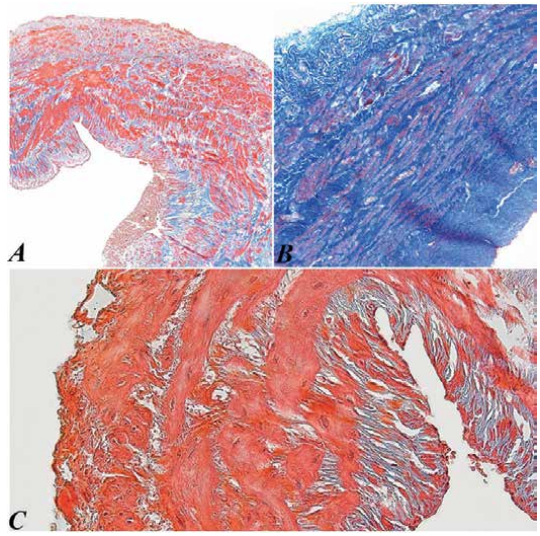

Figure 1.
Most of the study cases showed a significant infiltration at the vascular wall, and in some cases, the intima appeared thicker than media (A, original magnification x100). Thickening of the tunica media was observed, with collagen deposits and fibrous tissue between muscle fibers (C, original magnification x200). Also, Masson's trichrome staining showed subendothelial collagen deposition with reduced intimal smooth muscle cells (B, original magnification x200).

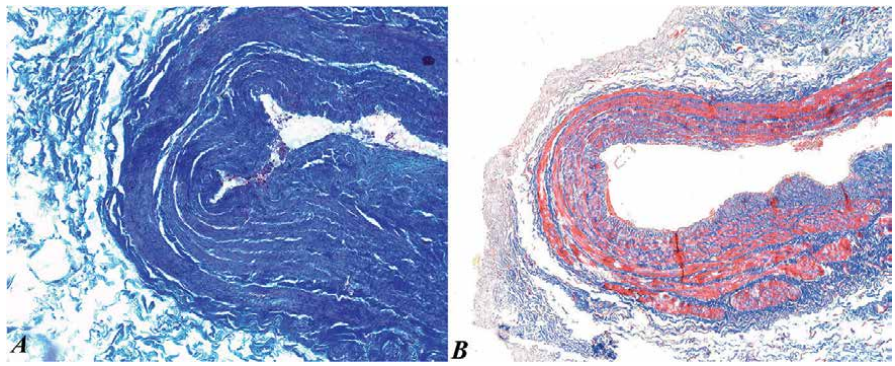

Figure 2.
In the control group, most of the cases showed moderate intimal thickening, with medial thickening through collagen deposits and hypertrophy of muscle fibers (A, original magnification x200). In some cases, muscle bundles were disorganized, with fragmentation of elastic fibers in the media, and absence of vascularity in the adventicea (B, original magnification x100).

a thickening of the TI correlated with the increase of collagen in the TA was also identified. The last correlation found in this study was the one between the maximum diameter and the wall thickness (0.69). Although a correlation is observed between these two parameters, it should be taken into account that no statistically significant differences were observed between groups, except in terms of the maximum venous diameter. Correlations are presented in **Table 2**, the relevant values being highlighted.

Regarding short-term postoperative follow-up, the following complications were encountered: bruising <2 cm surface in 9 cases (18%) in the SG and 12 cases (23.07%) in the CG; bruising = 2–10 cm in 5 cases (10%) in the SG and 9 cases (17.30%) in the

code	1	2	3	4	5	6	7	8	9	10
Study group										
1	1									
2	0.1804	1								
3	−0.1943	−0.1415	1							
4	0.0550	−0.2736	**0.6914**	1						
5	−0.0863	−0.1319	0.2560	0.4678	1					
6	0.2526	0.0650	−0.2936	0.0565	0.2721	1				
7	−0.1827	−0.2423	0.4113	0.3696	0.25	0.1360	1			
8	−0.0807	−0.1920	0.5285	0.3234	0.1666	0	**0.6667**	1		
9	−0.0700	−0.2887	0.3570	**0.8347**	**0.6123**	0.1666	0.4082	0.1020	1	
10	—	—	—	—	—	—	—	—	—	1
Control group										
1	1									
2	0.1323	1								
3	−0.0033	0.1677	1							
4	0.0205	0.2445	0.3350	1						
5	−0.0065	−0.1368	−0.0544	0.0413	1					
6	0.2477	0.0177	0.0628	0.1978	0.1494	1				
7	−0.0268	−0.0982	0.1254	0.2721	0.1805	0.4612	1			
8	0.2212	0.0822	0.2704	0.1368	0.1869	0.3768	**0.5728**	1		
9	−0.0396	0.1262	0.2503	0.2040	0.0038	0.1332	0.3514	0.3629	1	
10	0.1926	0.0507	0.1112	0.0350	0.1355	0.1689	0.3662	0.4482	0.3374	1

Table 2.
*Correlations between the analyzed parameters (1–10) * for the study and control group (Pearson correlation coefficients)* 1- Age (years); 2- BMI; 3- Maximum diameter (cm); 4- Wall thickness (cm); 5- Thickening of TI; 6- Thickening of TM; 7-Disorganized muscle bundles on TM; 8- Fragmentation of elastic fibers in TM; 9- Collagen increased in TA; 10- Absence of vasa vasorum in TA.*

CG; haematoma in one case (2%) in the SG and 2 cases (3.84%) in the CG. Although the proportions in which these complications were observed vary between the two groups, no statistically significant differences were highlighted. However, due to the slightly reduced number of these complications in the SG, it was associated with a shorter hospitalization period (average = 1.34 days) compared to the CG (average 1.75 days), statistically significant difference ($p = 0.002$). Regarding postoperative analgesia, no statistically significant differences were observed between the two groups regarding the type and quantity of analgesic drugs needed in postoperative pain management.

Overall, the postoperative outcomes were favorable in both groups. At the one-year evaluation, recurrent varicose veins presented as unsystematized varicose veins objectivable in the operated limb in clinical setting, were identified in two cases from the CG and in no case from the SG. However, no statistically significant differences were highlighted between the two groups from this point of view.

4. Discussions

Only the venous segments that showed reflux during duplex examination were surgically removed; therefore, the maximum diameters of the veins analyzed in this study refer strictly to these specimens. At the same time, we must take into account the fact that the surgically removed specimens were fixed in formalin solution, which led to a contraction of the veins and a decrease in their diameter through the action of the solution on the tissues. However, given the fact that all specimens were collected, prepared, and stored in the same way, the results obtained faithfully reflect the differences that appear between the study groups.

There are literature data which claim that the thickness of the GSV wall may increases with age, and it could be also slightly influenced by patient's gender [12, 13]. Although this study included patients with a wide range of ages, and the groups were made up of patients of both sexes, we did not identify statistically significant correlations between these parameters, the thickness of the venous wall, or the total diameter of the vessel.

In accordance with literature data, analyzing the control group, it was observed that CVD is associated with an increase in venous diameter [14]; a thickening of venous TI and TM [15]; that collagen content and thickness of the wall were increased at the level of the proximal segments compared to the distal ones [16]. However, the predominant damage to small-caliber vessels (collateral and perforating veins) which was noted in the study group, as well as the close correlation between the thickness of the venous wall and collagen deposits in the adventitia in these patients, suggest that these aspects could be related with the atorvastatin treatment.

Having a high efficiency in reducing serum cholesterol, current data suggest that atorvastatin is one of the most frequently used drugs in the treatment of dyslipidemias [17, 18]. Most frequently in current clinical practice, doses of 10–40 mg are used; higher doses being rarely used [19]. For the uniformity of the study group, only patients treated with doses of 10 mg of atorvastatin per day were included in this research. In this sense, a future direction of research aimed at prospective and randomized studies on larger groups of patients, would be useful to evaluate the evolution of CVD and the subsequent changes that occur at the level of the venous wall in patients treated with higher doses of atorvastatin, or with other statins; those studies will be useful for a comparative analysis and a better understanding of how these drugs have an effect on phlebological diseases.

There are literature data which claim that the increased number of apoptotic cells in the venous wall structure in patients with chronic venous insufficiency phenomenon were noted mostly in the proximal lower limb vein specimens, and could be involved in the final fibrosclerotic process acceleration, a main characteristic of the varicose vein wall [20]. Even if with the appearance of irreversible changes occurring in the more advanced stages of CVD (CEAP class >3) a decrease in inflammatory markers can be observed [21], as a result of the resolution of repeated inflammatory phenomena and fibrosis [22, 23], the inflammatory component remains one of the main factors incriminated in the emergence of this disease [23–25]. Due to the anti-inflammatory effect of statins on the vascular wall [8], as in the case of similar substances that reduce inflammation in the venous endothelium such as sulodexide [25, 26], the progression of CVD seems to be slowed down. This statement is supported by the observations of our study which proves a predominant impairment of the smaller veins within the study group.

At the same time, the anti-inflammatory properties of statins associated with their vasculotrophic and vasomotor effects [8] can explain the clinical observations, which highlight a rapid favorable postoperative evolution, including a decrease in hospitalization time, and a much lower complications rate. The fact that the absence of vasa vasorum in TA was not observed in any case of the study group, correlated with the data from the literature that prove the beneficial impact of statins exerted on blood vessels [8] are arguments that support the hypothesis that limiting CVD only to small caliber vessels with local fibrosis could be a result of atorvastatin treatment. These observations suggest that statin treatment may have a role on venous tone and trophicity. By improving the nutrient blood supply in the venous wall through vassa vasorum vessels, the vein tone is maintained for a longer time, and by this way, the progression of CVD could be delayed.

Moreover, there are several other studies that prove beneficial clinical effects of statins on various venous pathologies. A study conducted by Evangelista et al. showed that in the management of venous ulcers, simvastatin 40 mg daily in addition to standard wound care and compression is associated with a significant improvement in healing rate and time, as well as a better QoL for these patients [27]. There are several more studies in this field of investigation that support this hypothesis [28]. Furthermore, addition of simvastatin to standard therapy for the prevention of variceal rebleeding does not reduce rebleeding, but increases survival in patients with liver cirrhosis [29].

Additionally, statins seem to also impact venous thromboembolism. Lipid lowering therapy is associated with decreased venous thromboembolism risk after adjusting for known risk factors [30]. Several studies recommend that statins may be an alternative to anticoagulant treatment in thrombus formation and embolic events prevention [31, 32]. Considering the fact that deep venous thrombosis is one of the most frequent etiological factors responsible for CVD occurrence [33], and superficial venous thrombophlebitis is associated with the CVD progression as well [34, 35], the effects of statins may explain the observations related to the SG. Intraluminal thrombi where noted in 2.25 times lower percentage in the SG (4 patients) comparing with CG (9 patients). Those observations may be the result of the statin treatment and its beneficial effects on the circulatory system, and implicitly, on the morphology of the venous wall.

Overall, the results of this study suggest that atorvastatin treatment provide additional benefits in patients with CVD, this medication bringing anti-inflammatory, anticoagulant, phlebotonic and phlebotrophic effects. According to this study results, minimally invasive surgical procedures for phlebological patients that combine statin medication should be considered. In those cases, ASVAL technique could be performed for reflux veins excision [36]. The preservation of GSV was described in several papers [37–39]; if during clinical follow-up venous reflux is noted, venous ablation could be achieved by intravenous procedures [40], such as foam sclerotherapy [41], intravenous laser treatment [42], or VenaSeal. Preservation of the GSV can be considered a useful aspect in this category of patients under the conditions of a potential need for a venous graft for a vascular bypass, also.

5. Conclusions

Atorvastatin treatment seems to play a role in the morphological modifications of the venous wall associated with CVD, leading to increased collagen deposits and

a relatively concentric parietal thickening, affecting all venous parietal layers. In this category of patients, CVD expression is predominantly observed in smaller vessels, suggesting that the disease progresses slowly to larger venous trunks. The anti-inflammatory role of statin medication can be considered beneficial for the surgical phlebological patient. Moreover, the results of the study suggest a potential phlebotonic and phlebotrophic effect of statins. However, despite the fact that at first sight, the results of this study suggest that in this category of patients, minimally invasive procedures and conservative approach for GSV and SSV may be recommended, long-term clinical follow-up and further studies on larger number of patients are needed to determine if statin drug treatment could be considered as a decisional factor in CVD management.

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Conflict of interest

A part of this work, which represents the initial stage of this study, was published in "Phlebology: The Journal of Venous Disease," Volume 37 Issue 3, April 2022, as a research article entitled "Impact of statin treatment on patients diagnosed with chronic venous disease." "Morphological analysis of the venous wall and clinical implications". The initial article was cited accordingly.

The author has no other conflicts of interest to declare.

Abbreviations

CVD	chronic venous disease
CEAP	Clinical, Aetiological, Anatomical and Pathological Classification
LDL	low-density lipoprotein
SG	study group
CG	control group
BMI	body mass index
TI	tunica intima
TM	tunica media
TA	tunica adventitia
GSV	great saphenous vein
SSV	small saphenous vein

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Chapter 4

Statins and Epigenetics: A Putative Mechanism for Explaining Pleiotropic Effects

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Abstract

Statins remain the most efficient hypolipidemic agent and their use is pivotal in primary, secondary, and tertiary treatment of cardiovascular disease, reducing both morbidity and mortality. Statins target 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes conversion of HMG-CoA to mevalonate, the "committed and rate limiting step" in hepatic production of cholesterol. Genetic predilections for hypercholesterolemia are known to be responsible for substantial morbidity and mortality from cardiovascular disease. Environmental or lifestyle factors such as dietary fat and carbohydrate may also contribute to cardiovascular disease mortality by both genetic and epigenetic mechanisms. Besides lipid-lowering, statins have pleiotropic effects which may contribute to their protection against cardiovascular and several other diseases wherein hypercholesterolemia is a risk factor. Evidence is emerging that the clinical outcomes of many diseases are improved when modifications of environmental or lifestyle factors play integral roles in treatment and preventive prescriptions. This chapter is, therefore, intended to inform physicians and other health care professionals about the environment-gene interactions underlying the main and pleiotropic effects of statins which may be employed to improve the efficacy of statin therapies.

Keywords: cardiovascular disease, inflammation, HMG-CoA reductase inhibitor, pleiotropy, atherosclerosis, DNA methylation, histone acetylation, epigenetics

1. Introduction

The American Heart Association reported in 2018 that about 92.1 million American adults (i.e., more than one in four persons) had a cardiovascular disease [1]. This prevalence rises progressively with age, from 6 percent at age 20 to 77 percent at age above 75 years. Cardiovascular diseases have claimed more lives than all forms of other diseases and is responsible for 40 percent of all deaths, almost 1 million each year. Among all the cardiovascular diseases, coronary heart disease (CHD) remains

the leading cause of death by 43.8 percent, followed by stroke which accounts for 16.8 percent, high blood pressure 9.4 percent, heart failure 9 percent, arterial diseases 3.1 percent and other cardiovascular disease account for 17.9 percent [1]. The annual total costs of cardiovascular diseases are estimated to be more than $329.7 billion, both direct costs and indirect costs in lost productivity. Hence, there is a need to explore novel ways to decimate this disease burden.

An elevation in the concentration of some unhealthy blood lipids have shown to contribute largely to the pathogenesis of several cardiovascular diseases [2]. Hence combination of appropriate lifestyle changes and drug therapy can result in a decline of mortality rate caused by several cardiovascular events by 30–40% [3, 4]. One of the commonly used drugs is statin. It comprises a class of medications prescribed to treat patients with elevated low-density lipoprotein (LDL) by lowering cholesterol synthesis and promoting LDL catabolism [5]. Statins work by inhibiting the synthesis of cholesterol in the liver through inhibition of HMG-CoA reductase enzyme, which is known for driving the first committed and rate limiting enzymatic step in cholesterol synthesis [4]. Competitive inhibition of this enzyme reduces cholesterol synthesis and, ultimately, the circulating plasma concentrations of cholesterol. HMG-CoA reductase activity is controlled by several mechanisms: (1) rate of synthesis of HMG-CoA; (2) translation of HMG-CoA; (3) degradation of HMG-CoA; and (4) phosphorylation of HMG-CoA. This reduction in plasma cholesterol accounts for the therapeutic benefit of statins in reducing atherogenesis, plaque stabilization, and inhibiting thrombus formation [5, 6]. Studies in patients' taking statin have revealed 34–37% reduction in major cardiovascular events and 14–29% reduction in overall mortality. The statin drugs include lovastatin, simvastatin, pravastatin, cerivastatin, atorvastatin, fluvastatin, pitavastatin, rosuvastatin, etc.

Statins can elicit secondary, pleiotropic effects beyond lowering the blood cholesterol level. Some of the pleiotropic effects of statins appear to be linked to epigenetic mechanisms [7, 8]. Medical epigenetics is the science of unraveling the link between environmental factors and changes in expression of the genes which make individuals more, or less, susceptible to diseases. During cellular differentiation and function, there are molecular signals that turn genes on and off as needed by reversibly modifying transcription of the DNA into messenger RNA (mRNA) and translation of the DNA by transfer RNA (tRNA) into proteins without impacting the sequence of the DNA such as occurs with mutagenesis. The two most studied mechanisms are methylation of the DNA and acetylation of the histone proteins which provide tightly coiled DNA (heterochromatin) with a structural framework making up chromosomes. MicroRNAs (miRNA) play roles in epigenetics by suppressing translation. DNA methylation involves transfer of methyl groups from S-adenosyl methionine to the DNA nucleotides, generally, for silencing transcription and therefore inhibiting or downregulating expression of a particular gene. Histone acetylation uncoils the heterochromatin form of DNA making it available for transcription as euchromatin. Histone acetylation works by activating or inhibiting histone acetyltransferases (HATs) and histone deacetylases (HDACs), thereby increasing, or decreasing, gene expression. Exposure to environmental stressors like toxins, pollutants, temperature, and even some dietary components also initiate epigenetic modifications which may even be passed onto the next generation of offspring when exposures occur in utero. Other epigenetic changes could be due to numerous disorders, aging, and drugs such as statins [9].

2. Epigenetic mechanisms of statins

Statins are implicated in modifying gene expression by the epigenetic mechanisms of histone acetylation, DNA demethylation and upregulation or downregulation of miRNA [10, 11]. Studies have shown that application of a statin to cell cultures increases acetylation of histones 3 and 4 (H3, H4) [12–14] and decreases the production of mRNA for HDACs [10]. Four different studies have demonstrated inhibition of the active sites of HDACs [10, 12, 14, 15]. This epigenetic modification mechanism by statin is purported to mimic the actions of some HDAC inhibiting anticancer drugs such as belinostat, chidamide and romidepsin [16]. Statins are also implicated in demethylation of DNA by stimulating DNMTs found in the promoter regions of tumor suppressor genes [10, 11]. One statin in particular, simvastatin, is shown to downregulate gene expression via the mechanisms of inhibiting histone methyltransferases (HMTs), and therefore demethylation, of histone H3 at lysine, abbreviated "K" which is the 27th amino acid of histone H3 (H3K27) [17]. This epigenetic modification by statins appears to mimic the actions of the DNMT inhibiting anticancer drugs decitabine and azacytidine [16].

2.1 Epigenetic mediated pleiotropic effects of statins on cancer cell lines

Most cancerous cells possess HDAC activity that tends to promote cellular proliferation [18]. In. that regard, lovastatin is shown to inhibit HDAC1, HDAC2 and HDAC3 with increased acetylation of histone H3 which, in turn, appears to increase expression of cyclin-dependent kinase inhibitor 1 (p21), an intracellular mediator of apoptosis [14]. Statins in combination with an HDAC inhibitor seem to increase apoptosis of cancer cells and to also decrease the toxic adverse inflammatory effects compared to administering the HDAC inhibitor as a single drug [19, 20]. Simvastatin is reported to alter expression of more than 400 miRNAs, further showing its anticancer potential [10]. Th anticancer effects of statins appear to be mediated by targeting mRNAs involved in cell-cycle arrest, cellular proliferation, and angiogenesis. Examples of the role of statins are MiR-182 which has been shown to be a potent down-regulator of the anti-apoptotic protein Bcl-2, thereby facilitating apoptosis. Upregulation of miR-612 exhibits anticancer activity by making cancer cells more responsive to chemotherapeutic agents, and miR33b has been shown to downregulate c-Myc, a proto-oncogene that regulates transcription [10, 21, 22].

2.2 Anti-inflammatory and anti-atherosclerotic pleiotropic effects of statins

Statins appear to have properties which increases their efficacy in preventing adverse cardiovascular events [5, 23]. These pleiotropic anti-inflammatory and anti-atherosclerotic effects of statins involve the following mechanisms: optimizing endothelial functions [5], reducing metalloproteinase production in the extracellular matrix of endothelial cells which, in turn, increases the production of the main mediators of vasodilation such as nitric oxide [24, 25], inhibition of platelet aggregation, promoting atherosclerotic plaque stabilization by decreasing vascular inflammation [26, 27], enhancing myocardial parasympathetic responsiveness [28], moderating autonomic myocardial stimulation thus increasing myocardial perfusion [28, 29], angiogenesis upregulation, reducing inappropriate cardiac remodeling, upregulating baroreceptor sensitivity [30], downregulating

cerebral vasospasm [31, 32], and reduction in expression of the angiotensin II type I receptor mediating vasoconstriction [33].

2.3 Molecular and epigenetic basis for the anti-inflammatory and anti-atherosclerotic properties of statins

Statins are implicated in activation of epigenetic mechanisms that increase the acetylation of histones 3 and 4 which, in turn, decrease the activity of macrophages [12]. Macrophages are the main immune cells found in atherosclerotic plaques and are, therefore, the main determinants of the inflammatory and atherosclerotic potential of such plaques. During cholesterol synthesis, there are lots of isoprenoid intermediates produced [25] such as the isoprenes geranylgeranyl and farnesyl. These intermediates are important for post-translational modification of proteins in order to covalently bind some proteins and traffic them to membranes where they function. Protein isoprenylation occur mainly on proteins containing C-terminal cysteine aliphatic amino acid (CaaX motif), and some members of the Ras and Rho GTPase family are involved in isoprenylation [23, 25]. The significance of this is that GTPases are involved in regulating cytoskeletal and intracellular signaling traffic pathway by alternating between active and inactive GTP-bound states. As a result of this alternation, they regulate cellular growth, migration, morphogenesis, and cytokine trafficking. The Rho GTPase family consist of RhoA, Rac, and Cdc42, they are known for regulating cell cycle progression, proliferation, vesicle trafficking, cell shape, and maintaining optimal microtubule functions. Some Rho associated protein kinases are involved in hypertension by modulating calcium-insensitive vascular smooth muscle contraction and coronary spasm [25]. Statins have been reported to inhibit proliferation of smooth muscle cells in the arterial wall by inhibiting RhoA isoprenylation in these cells, thus distorting membrane trafficking and gene transcription. This leads to alteration in the actin cytoskeleton and inhibition of assemblies of proteins for focal adhesion, thus enhancing endothelia cell functions [34]. An epigenetic study speculates that the potential for inhibiting Rho signaling is linked to statin induced upregulation of miRNAs [35].

Rac is another GTPase involved in atherosclerosis, known to promote inflammation by generating reactive oxygen species, by activating NADPH oxidase activity, and by binding cytoskeletal remodeling proteins like p21-activated kinase and calmodulin-binding GTPase activating proteins. Statins are shown to inhibit Rac mediated NADPH oxidase activity, thereby reducing the free radical production resulting from angiotensin II and protecting against endothelial dysfunction, as well as cardiac and smooth muscle hypertrophy [36, 37]. Statins are also shown to increase histone acetylation and expression of angiotensin-converting enzyme 2 (ACE2), thereby counterbalancing the effects of angiotensin II and indirectly ameliorating risk factors for the development of atherosclerosis [37].

The hypolipidemic effects of statins are likely responsible for the main mechanism of protection against endothelial dysfunction by reducing plasma lipid particles, which, in turn, upregulates endothelial nitric oxide synthase (eNOS) and production of nitric oxide [38]. This appears to be achieved by prolonging the half-life of eNOS with involvement of PI3K/Akt activation, RhoA geranylgeranylation, and inactivating an integral membrane protein responsible for binding eNOS in caveolae [39]. The activation of eNOS leads to reduction in ROS and deactivation of pro-inflammatory transcription factors. Statins are also shown to cross-react with sphingosine-1-phosphate (S1P) a naturally occurring bioactive lysophospholipid

in G-protein-coupled S1P receptors, thereby regulating cell-to-cell and cell-to-matrix adhesion, cell migration, differentiation, and survival of endothelial cells [40]. Such effects of statins on SIP receptor mechanisms are likely to enhance endothelial responsiveness to HDL, thereby inhibiting lipid oxidation for invasion and deposition, promoting lipid transport to the liver for degradation and clearance from the circulation and, ultimately, improving endothelial function. However, the involvement of statin induced upregulation of histone acetylation and miRNAs in these lipidemia-related effects remains unknown.

High sensitivity C-reactive proteins (hsCRP) synthesized in the liver as acute phase reactants are elevated in patients with increased risk for stroke and heart attack [4]. CRP is produced in response to pro-inflammatory cytokine released during atherogenesis, thus binding to modified LDL particles within the plaques and eventually activating complement protein that enhance inflammation. Lovastatin decreases plasma levels of C-reactive protein thus reducing risk for acute coronary events in patients with relatively low plasma LDL cholesterol levels [4]. DNA methylation of CRP is an epigenetic mechanism shown to be correlated with cognitive decline (41). Because of the strong connection between cognitive decline and atherosclerosis. Whether DNA methylation patterns are correlated with atherogenesis independently of cognitive decline, should be investigated.

Studies have also shown that statins influence the regulation of endothelial progenitor cells (EPC) which play a vital role of repairing and angiogenesis at the site of vascular damage or ischemia especially in patients with coronary and peripheral vascular diseases. This process is thought to be mediated by multiple miRNAs, thus promoting the release of cytokines and endothelial cells differentiation. A clinical trial involving patients with cerebrovascular disease taking atorvastatin for eight months reported downregulation of miR34a and increased EPC blood counts [41]. A similar finding was reported in another study demonstrating downregulation of miR-221 and miR-22 expression and increased EPC peripheral blood counts after 12 months of atorvastatin treatment [42].

Other anti-inflammatory properties of statins include decreased expression of TNF-alpha and IL-1β from macrophages eventually downregulation of mononuclear leukocyte proliferation in the blood [43]. There is also inhibition of β2-integrin antigen-1, thereby decreasing lymphocyte adhesion molecules and the secretion of anti-inflammatory Th2-type cytokines [44]. A randomized control study performed by giving 40 mg of simvastatin daily to patients (n = 25) or placebo (n = 20) for two months demonstrated reductions in serum inflammatory markers, such as IL-6, IL-13, IFN-γ, IP-10, MCP-1, and VEGF [45]. There also is evidence from animal studies that rosuvastatin may increase the expression of CC-chemokine receptor 7 (CCR7), a key regulator of macrophage emigration. Crosstalk between DNA methylation and numerous pro-inflammatory and anti-inflammatory cytokines has been reported (46). These findings appear support the main hypothesis of the present review that epigenetic mechanisms are likely to play a critical role in atherogenesis.

3. Environment: gene interaction for statins in cardiovascular regulation: emphasis on autonomic balance and baroreceptor sensitivity

Statins have the potential of sympathetic activity modulation in the heart and great vessels, coupled with an increase in baroreceptor sensitivity. Some underlying mechanism involve increase in endothelial nitric oxide synthase (eNOS) thus

improving endothelial function and baroreflex control of blood pressure from the central nervous system. Moreira et al. corroborated statin's ability to enhance aortic depressor nerve activity and better arterial distensibility by increasing the number of carotid elastic lamella with resultant reduction in vessel intima thickness. Reducing the mechanical stress on the walls of the carotid and aortic arteries plays vital role at baroreceptor sensitivity enhancement [46]. Angiotensin 1 receptor (AT-1) and nicotinamide adenine dinucleotide phosphate oxidase are also modulated by statins thus potentiating the ability of reducing peripheral sympathetic activity [47]. There is evidence that oral atorvastatin treatment in hypertensive rat model improves sympathovagal balance by reducing reactive oxygen species in the rostral ventro-lateral medulla and increasing eNOS expression in the nucleus tractus solitarius [48].

Tetraspanin 2 protein (TSPAN2) vastly expressed in vascular tissue has been implicated in maintaining vascular smooth muscle contractile ability and its cellular differentiation. This protein is linked to large artery atherosclerosis-related stroke due to its proinflammatory, highly proliferative and migratory tendencies. An epigenome-wide association study identified that 0.1% decrease in DNA methylation at cg23999170 results in increased TSPAN2 expression thus leading to a 5 mmHg increase in diastolic blood pressure [49]. TSPAN2 signaling is regulated by TGFB1/Smad. TGFB1/Smad signaling is a target of statins for preventing atherogenesis. These findings implicate statins as a potential mediator of changes in DNA methylation associated with the expression of TSPAN2. Future studies should determine whether statins induce changes in DNA methylation which may protect arteries from atherogenesis by increasing the expression of TSPAN2 and from hypertension by increasing baroreceptor sensitivity. These findings seem to support the concept of crosstalk between epigenetic regulations of vascular structure and baroreceptor sensitivity for protection against both atherogenesis and hypertension.

4. Environment: gene interaction for statin-induced type 2 diabetes

Long term statin use especially at higher doses has been shown to increase the risk of users developing type-2 diabetes [50]. This stems from impaired secretion of insulin from the beta-cells of the pancreas, decreased insulin sensitivity, and poor utilization of insulin at the peripheral tissues. Some of the epigenetic modification linked to statin therapy includes statin potential to methylate DNA and alter miRNA expression which are known to play key role in the regulation of glucose and lipid metabolism. miRNA silences gene expression, thus affecting insulin expression, insulin sensitivity, and skeletal muscle adaptation to elevated glucose. Several studies have revealed that statin treatment can upregulate miRNA-33 family (33a and 33b) expression, which plays significant role in statin's pleiotropic effects [22, 51].

MiRNA-33a encodes the introns of SREBP1 gene thus targeting the export of cholesterol, fatty acid metabolism, high density lipoprotein (HDL) regulation, inhibiting ABCA1 and ABCG expression. ABCA1 plays a vital role at regulating beta-cell activity in the pancreas. Inhibiting expression of such gene may cause beta-cell dysfunction due to alteration in islet cholesterol homeostasis and impaired insulin secretion [52]. miRNA-27 family have also been implicated in downregulation of low-density lipoprotein receptor-RNA (LDLR-RNA), LRP6, LDLRAP1, and indirectly upregulating PCSK9 [53]. Alteration of these proteins negatively impacts the correct binding

of clathrin endocytosis of LDLR-LDL-C complex thus resulting in LDLR inefficiency and subsequently insulin resistance.

Increase in hepatic glucose production has been seen as one of the direct pleiotropic effects of statin mediated upregulation of miRNA-183/96/182 cluster [54]. Thus, resulting in higher expression of key gluconeogenic enzymes like phosphoenol pyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) which may contribute to developing type-2 diabetes due to poor glycemic control. Essentially, when insulin binds to its receptor (insulin receptor- INSR), it triggers cascade of events by modulating PI3K/PDK1/Akt pathway. This signaling therefore result in metabolic effect that helps normalize blood glucose level. Upregulating some of these miRNAs negatively impact the expression of insulin receptor substrates-2 (IRS2) which may potentially affect insulin signaling and end up contributing to risk factors for insulin resistance. Also, simvastatin's downregulation of miRNA-146a inversely alter the expression of protein tyrosine phosphatase non-receptor type 1 (PTPN1) which is known to negatively affect regulation of insulin signaling [55].

Other mechanisms through which statin predisposes one to type-2 diabetes includes limiting glucose uptake in peripheral cells. It downregulates glucose transporter-4 (GLUT-4) expression at the plasma membrane thus impairing insulin signaling. There is also statin-induced isoprenylation inhibition, Rab-4 and RhoA isoprenoid-dependent proteins which are known to aid insulin-induced translocation of GLUT-4 are impaired thus inefficient insulin signaling. It can also induce changes in hormones like leptin and adiponectin, and free fatty acid circulation. Insulin secretion from the pancreatic beta cell is mostly initiated by glucose induced ca2+ entry which is controlled by voltage gated L-type calcium channel. Simvastatin in rat model has shown to impair this channel activity thus resulting in an impairment of insulin secretion. GLUT-2 mRNA and protein expression is also inhibited by statin at the beta cells of the pancreas thus limiting the glucose uptake and metabolism.

The metabolic syndrome in men study (METSM) found 24% insulin sensitivity reduction, 12% reduction in pancreatic beta cell reduction, and 46% increase in the risk of developing type-2 diabetes mellitus (T2DM) and worsening hyperglycemia when compared to men no on statin treatment [56]. This side effect of statin portends higher risk in patients with pre-existing risk factors like higher body mass index (BMI) or HbA1c, or impaired fasting blood glucose). It is interesting to note that atorvastatin and simvastatin elicit these effects in a dose dependent fashion.

5. Final discussion

The duration of statin use that is likely to result in primary or secondary prevention of cardiometabolic events is not clearly known. But it has been observed that the lipid lowering property of statins may require 6–24 months of treatment while the endothelial-dependent vasomotor effects of statins appear to be more rapid, about 6 months from the initiation of treatment [57]. It is noteworthy that when vasodilators are given, the potential inhibition of pro-thrombotic mediators may increase stabilization of the atherosclerotic plaque and, thereby, prevent thrombotic complications. Whether these benefits are epigenetically mediated remain open to debate. Statins appear to have pleiotropic anticancer properties in vitro, as reflected in lower

rates of cancers in patients using statins [58]. On the other hand, meta-analyses of clinical trials report no such anticancer effect [59]. These contradictory reports could, partly, be attributed to different statins having different biochemical properties. For example, treatment with a hydrophilic statin, pravastatin, was associated with less DNA methylation of cancer cells compared to a more lipophilic statin, simvastatin [11]. Simvastatin is reported to downregulate the expression of histone-lysine-N-methyltransferase known as EZH2, whereas pravastatin had no effect on EZH2 [17]. On the other hand, simvastatin is reported to downregulate the expression and activity of DNMTs and HDACs [15], whereas lovastatin only reduced the activity of both these enzymes without altering their expression [15]. It is noteworthy that these epigenetic mechanisms may not be connected to the therapeutic effects of statins based on evidence which supports the notion that statins can produce effects by mechanisms involving changes in phenotype and/or cell metabolism, as reported for prolonged use of metformin [60]. Similar observations have been made in the outcome of lupus patients in whom the CRP levels were correlated with the use of a particular statin; plasma CRP in lupus patients using lipophilic atorvastatin was found to be significantly lower than the CRFP levels associated with use of rosuvastatin or pravastatin [61]. **Table 1** recapitulates statins' potential epigenetic mechanisms and effects.

Potential pleiotropic effect	**Epigenetic mechanism**	**Potential molecular response**
Anti-atherosclerosis: Enhancing endothelial function	• ↑ histone acetylation • ↑ DNA methylation • upregulation of miRNAs • miR-221 and miR-222 downregulation • miR-34a downregulation	• ↑ expression of ACE2 and CCR7 • ↓ TSPAN2 expression • ↓ RhoA signaling • ↑ blood plasma EPC counts • ↑ expression of HDAC1, HDAC7, and SIRT1
Baroreceptor sensitivity enhancement	↑ DNA methylation	↓ TSPAN2 expression
Potential for anti-cancer activity	• ↓ DNA methylation • Inhibition of HMT • miR-182 upregulation • miR-33b upregulation • ↑ histone acetylation	• ↑ expression of BMP2 • ↑ expression of p27 • ↓ anti-apoptotic protein Bcl-2 • ↓ proto-oncogene c-myc • ↑ expression of p21 (tumor suppressor gene), HDAC inhibition
Increased risk for Type-2 diabetes	• miRNA-33a and miRNA 33b upregulation • miRNA-27 alteration • upregulation of miRNA-183/96/182 cluster. • downregulation of miRNA-146a	• ↓ ABCA1 and ABCG expression in pancreas beta cells • downregulation of LDL-RNA, LRP6 and upregulation of PCSK9 • ↑ expression of G6Pase and PEPCK • Altered expression of PTPN1 and poor insulin signaling.

Table 1.
Summary of potential epigenetic modification induced by statins and their pleiotropic effects.

6. Conclusions

This chapter provides insights to how primary and secondary effects of statins could be mediated by epigenetic modifications, via direct and indirect mechanisms. Statins are a highly effective class of drugs used to treat hyperlipidemia, with more than 30 million users in the United State alone. Besides the lipid lowering activity of statins, it has also been demonstrated from several studies that statins have anti-inflammatory properties leading to atherosclerotic plaque stabilization and enhancement of endothelial function. These therapeutic benefits appear to decrease susceptibilities to thrombus formation and overall reduction in mortality from cardiovascular events. Overall, statin use as an adjunct to other standard-of-care drugs may have the potential to reduce cholesterol levels, induce immunomodulation, anti-inflammation, neuroprotective effects, alleviate chronic kidney disease progression, improve vascular function and bone metabolism. Despite this growing body of evidence, there is a need to quantify the pleiotropic effect of statins on specific genes or pathways to fully appreciate the use of statin as a stand-alone drug or in synergy with other drugs.

The pleiotropic effects of statins are evidenced mainly by anti-inflammatory properties which maintain a homeostatic balance between pro-inflammatory and anti-inflammatory mediators. This balance protects against the endothelial dysfunction associated with hyperlipidemia and cardiovascular disease. Overall, the pleiotropic effects of statins include: reduction of metalloproteinase production in the extracellular matrix, increased production of vasodilatory mediators like nitric oxide, inhibition of platelet aggregation, promotion of atherosclerotic plaque stabilization, enhancement of myocardial parasympathetic responsiveness, moderation of myocardial sympathovagal balance, increased myocardial perfusion, upregulation of angiogenesis, inhibition of cardiac remodeling, upregulation of baroreceptor sensitivity, downregulation of cerebral vasospasm, and reduction in expression of angiotensin II and angiotensin-1 receptor type I. The mechanisms through which statins elicit these secondary effects have been extended to the level of epigenome, albeit with little systematic investigation of epigenetic mechanisms and effects. With these promising effects of statins, this therapeutic review therefore calls for more clinical trials in human population across several cardiometabolic diseases to harness these potential benefits. Healthcare practitioners are also to be aware that the inherent ability of statins to recruit epigenetic modifications like acetylation of histones, upregulation or downregulation of micro ribonucleic acid, and methylation of DNA contribute to the pleiotropic effects of statins, while it's not just limited to cardiovascular disease, there are potential application to various other diseases as well.

Conflict of interest

The authors declare no conflict of interest.

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Section 2

Statins' Effects beyond Cardiovascular System

Chapter 5

Statin Therapy and Gut Microbiota

Peng Chen and Kangbao Li

Abstract

Accumulating studies reveal that statins are associated with distinct gut microbiota profiles. Statin therapy helps maintain gut microbiota homeostasis, reducing the prevalence of gut microbiota dysbiosis and breaking antimicrobial resistance. The possible mechanisms may include improving bile acids metabolism, regulating intestinal innate immunity, and inhibiting cell membrane biosynthesis. Statin treatment might benefit patients with obesity, cardiovascular diseases, malignancies, and immune-related diseases by modulating the compositions and functions of gut microbiota. The altered gut microbiota functions by regulating the host metabolism with microbial-derived metabolites, such as primary and secondary bile acids (BAs) and short-chain fatty acids (SCFAs). Meanwhile, statins can be degraded or modified by the gut microbiota, which may affect the treatment effectiveness in clinic. The addition of probiotics could enhance the effects of statins on hypercholesterolemia and inflammation. Collectively, the interaction between statins and gut microbiota shows great promise for new therapeutic targets and personalized medicine in many diseases, which still need further investigation.

Keywords: statins, gut microbiota, host metabolism

1. Introduction

Our intestinal tracts harbor countless microbes, considered as a new organ for their essential effects [1]. Gut microbiota regulates the occurrence and development of many kinds of diseases, including gastrointestinal disease, neuropsychiatric diseases (gut-brain axis), metabolic disorders, cardiovascular disease, infectious diseases, and malignant tumors. The related mechanisms include pathogen defense, maintaining mucosal homeostasis, interaction with immune system, and participation in human metabolic processes. Gut microbiome composition is influenced by age, diet, antibiotic drugs, and other environmental exposures [2]. Notably, gut microbiota can also interplay with nonantibiotic drugs, resulting in altered microbiota composition or changed drug effectiveness [3]. Microbiome-based therapeutics, such as fecal microbiota transplantation (FMT), have become promising for several diseases associated with changes in gut microbiota [4]. The concerns about the interaction between microbes and drugs may limit their practical use. Statins are the inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-R), a rate-limiting enzyme in the cholesterol synthesis pathway. Statins show a potent effect of lowering plasma lipid levels and are considered as protector against atherosclerosis, inflammation, oxidation, and thrombogenesis [5]. Meanwhile, accumulating studies

have also revealed that statins could regulate gut microbiota. Here, we aim to review the bidirectional interactions between statins and gut microbiota and the underlying clinical consequences.

2. The association between statin therapy and gut microbiota signature

Replace in a Dutch cohort of 1135 patients, statins were significantly associated with changes of β diversity [6]. Recently, Vieira-Silva et al. have divided gut microbial profiles into four enterotypes based on the abundance of signature species: Bacteroidetes 1 (Bact1), Bacteroidetes 2 (Bact2), Rumen cocci (Rum), and Prevotella (Prev). Bact2 enterotype is associated with inflammation, showing a high proportion of Bacteroides and a low proportion of *Faecalibacterium*. Patients with obesity who take statins show a lower prevalence of Bact2 than those who did not take statins (5.9 vs. 17.7%, $P < 0.01$), suggesting that statin therapy helped reduce gut microbiota dysbiosis [7]. Moreover, statins can alter the microbiome composition in patients with dementia, whose role in dementia remains to be elucidated [8].

Different statins show different influence on the microbiota. Atorvastatin promotes the relative abundance of anti-inflammatory microbiota, such as *Faecalibacterium prausnitzii*, and reduces the abundance of proinflammatory bacteria, such as *Desulfovibrio* sp., in patients with hypercholesterolemia [9]. In a rat obesity model induced by a high-fat diet (HFD), atorvastatin treatment restored the gut microbiota diversity with an increased abundance of Proteobacteria and a decreased proportion of Firmicutes [10]. And rosuvastatin mainly decreased the ratio of Firmicutes/Bacteroidetes [11–13]. Kim et al. found that rosuvastatin remarkably increases microbial diversity more than atorvastatin in HFD mice. FMT with fecal material collected from rosuvastatin-treated mice improves glucose tolerance and metabolic disorders and decreases inflammatory factor, IL-1β [11]. Martin et al. found that rosuvastatin had collective genetic changes of microbiota configuration for reduced transportation and metabolism of trimethylamine-N-oxide (TMAO) and increased betaine and gamma-butyl betaine [14].

Statins can show anti-bacterial effects to some extent. Statins help break antimicrobial resistance by acting synergistically with antibiotics to weaken virulence factors, boost the body's immunity, or help wound healing [15, 16]. Simvastatin had the highest antibacterial activity against Gram-positive bacteria compared with atorvastatin, rosuvastatin, and fluvastatin. Atorvastatin is generally similar to or slightly better than simvastatin against Gram-negative bacteria, but both are more effective than rosuvastatin and fluvastatin [17]. The mechanism of antibacterial activity of statins is likely to interfere with the regulatory function of bacterial cells by binding to and destroying cell surface structures [18, 19]. Nolan et al. reported that rosuvastatin can inhibit HMG-R (+) bacteria pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes* which was associated with a reduction in bacterial-induced mevalonate levels [20].

3. Mechanisms of statins in regulating gut microbiota composition

Since statins are potent cholesterol-lowering medicines, bacteria that depend on host cholesterol are inhibited directly [21]. Changes in bile acid metabolism

are the main mechanism affecting gut microbiota caused by statins. Bile acids, the main components of bile, are produced in liver cells by the conversion of cholesterol. Animal experiments showed that a high-fat diet increased bile acid excretion, leading to an increase in Firmicutes/Bacteroidetes ratios and a decrease in microbial richness and biodiversity [22, 23]. The composition and diversity of microbiota caused by bile acid differences were also found in populations with different diets [24]. The major regulatory enzymes catalyzing bile acid synthesis are Cyp27a1 and Cyp7a1 [25]. lslam et al. found that statins can inhibit bile acid biosynthetic pathway by down-regulating Cyp27a1 gene and inhibit the conversion of 7 alpha hydroxycholesterol [12]. Caparros-Martin et al. demonstrated that statins induced metabolic alterations through pregnane X receptor (PXR) pathway, which was responsible for the deregulation of Cyp27a1 and Cyp7a1. PXR deletion significantly attenuated the statin-induced changes in gut microbiota composition [26]. Additionally, Cheng et al. found that statin therapy may activate NF-κB signaling pathway, induce intestinal inflammation and change mucosal barrier function, which alters the composition of gut microbiota [27]. Accumulating studies have revealed statins can inhibit certain pathogenic bacteria, which partly accounts for the regulatory effect of statins on gut microbiota composition. The mechanism of their antibacterial activity has been described previously.

4. Influence of gut microbiota on the therapeutic efficacy of statins

Statins have been shown to influence gut microbial profile; in turn, the cholesterol-lowering effect of statins can be regulated by gut microbiota. Compared to mice with intact gut microbiota, antibiotics-induced abiotic mice did not respond to atorvastatin treatment with the altered expression of cholesterol-lowering genes (Ldlr, Srebp2, and Npc1l1) [28]. Simvastatin showed a similar effect, which was associated with genes regulating bile acids synthesis [29]. Additionally, Gut microbiota can mediate the production and degradation of active β-hydroxy acid form of lovastatin [30]. Furthermore, patients with higher gut biodiversity predict well respond to statins, showing lower levels of total cholesterol and LDL cholesterol (LDL-C). A significant increase in Bacteroides, *Holdemanella*, *Clostridium* and a decrease in *Lactobacillus*, and *Bifidobacterium*, predict a poor response to statins therapy with more adverse effects [31, 32]. The effect of *Faecalibacterium* on statins is still controversial [33, 34].

Microbiota-derived productions can mediate statins efficiency through several pathways. Baseline concentrations of microbiota-produced lithocholic acid, taurolithocholic acid, and glycolithocholic acid were positively correlated with simvastatin-related LDL-C reduction levels [35]. Jones et al. performed a randomized controlled trial and found that patients treated with *Lactobacillus reuteri*, a bacteria containing bile salt hydrolases, have significantly reduced LDL-C levels [36]. Zhang et al. revealed that Bacteroidaceae, Prevotellaceae, and Porphyromonadaceae-mediated the effect of simvastatin through phenylalanine and tyrosine-associated amino acid metabolism pathways [37]. Statins influence the concentration of microbiota-derived metabolites, such as short-chain fatty acid (SCFAs), TMAO, and lipopolysaccharides (LPS), which in turn mediate lipid metabolism by targeting PPARγ, TLR4-Myd88, FXR, and PXR signaling pathways [38].

5. The interaction between statins and intestinal microbiota regulate several diseases

Statins modulate gut microbiota which influences intestinal barrier function. Zhang et al. found that atorvastatin increased the abundance of Firmicutes and *Lactobacillus* and decreased the abundance of Bacteroidetes, and improved the mucosal barrier function (increased protein levels of tight junction protein), then alleviates microbiota-mediated neuroinflammation in ischemic stroke mice [39]. Moreover, antibiotic prophylaxis are not suitable for cirrhosis, in case of antibiotic resistance. Statins have been found to inhibit bacterial pathogens, boost intestinal innate immunity, and maintain intestinal barrier function, which can be novel strategies to prevent infections in cirrhosis [40]. Similarly, simvastatin can maintain intestinal integrity and inhibit bacterial translocations from gut lumen to blood circulation, which can improve the prognosis of endotoxemia [41]. Increased production of methane was found in patients with irritable bowel syndrome with constipation due to their inhibitory activity on gut smooth muscle [42]. Lovastatin can reduce methane production by directly inhibiting cell membrane biosynthesis of methanogenic archaea without affecting bacteria numbers and inducing microbiota dysbiosis [43].

The interaction of statins with gut microbiota in malignant tumors has been extensively investigated. *Helicobacter pylori* infection is known as a crucial risk factor for gastric cancer (GC). *H. pylori* virulence factors promote inflammation and tumorigenesis, which requires the utilization of host cholesterol. Statins were found to inhibit *H. pylori*-associated GC by regulating *H. pylori* virulence factors and ROS production [21]. Zhang et al. demonstrated that nonalcoholic fatty liver disease (NAFLD)-associated hepatocellular carcinoma (HCC) was associated with high cholesterol-mediated gut microbiota dysbiosis, that is, an increase in *Desulfovibrio*, *Mucispirillum*, *Anaerotruncus*, and Desulfovibrionaceae, and a decrease in *Bifidobacterium* and Bacteroides, while the administration of atorvastatin can prevent NAFLD-HCC significantly by restoring cholesterol-associated microbiota dysbiosis [44]. The anti-tumoural effect of checkpoint inhibitors may decrease when co-administration with proton pump inhibitors, glucocorticoids, antibiotics, and psychotropic drugs for their interaction with gut microbiota. However, baseline co-administration with statins was safe and did not affect patient prognosis [45].

Statin therapy may benefit patients with acute coronary syndrome (ACS) by regulating the composition and function of the gut microbiota. Statins regulate the gut microbiota of ACS patients in a healthier direction (characterized by an increased abundance of beneficial bacteria such as *Anaerostipes hadrus* and reduced abundance of potential pathogenic bacteria such as *Paracetobacterium merdae*) [46]. Additionally, Li et al. showed that statins can reduce the risk of major adverse cardiovascular events though reducing plasma TMAO levels derived from microbiota [47].

6. Conclusion

This review aims to summarize the bidirectional interaction between statin therapy and gut microbiota and to describe the underlying mechanisms. Statins regulate the composition of gut microbiota and change the microbiota-derived metabolites, which in turn influence the cholesterol-lowering effect of statins. However, inconsistent results have been reported regarding the altered gut microbiota profile in different studies, and most studies evaluating the effects of statins on gut microbiota

to treat diseases are currently in the preclinical stage. Further experimental studies and clinical trials are required to investigate personalized treatment based on the interaction between statins and gut microbiota.

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Conflict of interest

The author declares that there are no conflicts of interest.

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Chapter 6

Statins against Cancers: Role of Inhibition of Voltage-Gated Potassium Channels Kv1.3

Andrzej Teisseyre, Kamila Środa-Pomianek, Anna Palko-Labuz and Mateusz Chmielarz

Abstract

Statins are organic compounds, which are applied in medicine, basically to reduce blood cholesterol level. Studies performed during past years provided evidence that statins may also be applied in the therapy of some types of cancer, such as colorectal cancer, breast cancer, or leukemia. Anticancer activity of statins may be due to the inhibition of voltage-gated potassium channels Kv1.3. Inhibition of these channels may exert antiproliferative and pro-apoptotic effects on Kv1,3 channel-expressing cancer cells. This may lead to a selective apoptosis of the cancer cells while sparing the normal ones. This chapter focuses on the inhibitory effects of statins on Kv1.3 channels and on the antiproliferative and pro-apoptotic effects of these compounds on Kv1.3 channel-expressing cancer cells. It is shown that the statins lovastatin, mevastatin, pravastatin, and simvastatin are effective inhibitors of the channels expressed in cancer cell line Jurkat T. The channel inhibition may be related to the anticancer activities of these compounds. Moreover, pro-apoptotic activity of the compounds is significantly augmented upon co-application of the statins with flavonoids and xanthohumol. This may be related to an additive or synergistic inhibition of Kv1.3 channels in these cells by the compounds applied in combination.

Keywords: statins, flavonoids, Kv1.3 channel, cancer cell proliferation, cancer cell apoptosis

1. Introduction

Statins are organic compounds, which can be obtained from plants, such as *Aspergillus* fungi. To this group belong, among others, lovastatin, mevastatin, pravastatin, and simvastatin. Other well-known statins, such as atorvastatin, pitavastatin, and rosuvastatin, were obtained in a chemical synthesis.

Statins are known as inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which plays a crucial role in the biosynthesis of cholesterol [1]. Therefore, statins may widely be applied in the treatment of hypercholesterolemia and atherosclerosis. According to the recently formulated hypothesis, statins may also be applied in the treatment of severe COVID-19 cases. This may due to the inhibition of

cholesterol biosynthesis, which leads to a reduction of cholesterol content and prevention of SARS-CoV-2 virus's entry into host cells [2].

It is known that statins may exert pleiotropic effects, which are far beyond their ability for inhibition of biosynthesis of cholesterol. It is known that statins may also exert anticancer activities. It was shown that statins may exert antiproliferative and proapoptotic effects on many cell lines of many different types of cancers [1]. Among cancer cell lines, which are targets for statins, are breast cancer cells, colon carcinoma, glioblastoma, leukemia, melanoma, myeloma, pancreatic cancer, prostate cancer, and thyroid cancer [1]. The statins that are active as anticancer agents are: lovastatin, cerivastatin, fluvastatin, simvastatin, pravastatin, and atorvastatin [1].

Recently published data has provided evidence that the statins simvastatin and mevastatin exert antiproliferative and proapoptotic effects on human colorectal adenocarcinoma cell line LoVo and its doxorubicin-resistant subline LoVo/DX [3]. Importantly, application of the statins re-sensitized the LoVo/DX cells to doxorubicin treatment. Interestingly, the anticancer activities of the statins were significantly augmented when the statins were co-applied with flavonoids 6-hydroxyflavone, 7-hydroxyflavone, and baicalein [3]. It was shown that the statins and the flavonoids may act synergistically when co-applied with each other. The most significant synergism was observed upon the co-application of simvastatin with baicalein on LoVo/DX cells [3].

Flavonoids are a wide group of plant-derived compounds, which exert pleiotropic effect on many molecular targets. Among them are voltage-gated potassium channels Kv1.3, which are inhibited by some flavonoids, such as genistein, acacetin, chrysin, natural (prenyl), and synthetic (methoxy) derivatives of naringenin [4]. Similar inhibitory effects on the channels are exerted by some chalcones, such as xanthohumol [4] or isobavachalcone (Teisseyre – unpublished results).

Voltage-gated potassium channels (Kv) are integral membrane proteins, which are selectively permeable for potassium ions and are activated upon a change of the cell membrane voltage. Activation of these channels enables transportation of potassium ions across the cell membrane down the electrochemical gradient. The channels are known as "delayed rectifier" Kv channels, which activate upon membrane depolarization and undergo a slow and complex C-type inactivation [4, 5]. Activation of Kv1.3 channels in the plasma membrane provides an efflux of potassium ions out of the cell and stabilization of the resting membrane potential [5]. Kv1.3 channels are mammalian *Shaker* Kv channels, encoded by the KCNA3 gene [4, 5]. Kv1.3 channels are expressed abundantly in human T and B lymphocytes, macrophages, fibroblasts, platelets, macrophages, osteoclasts, microglia, oligodendrocytes, brain (e.g., olfactory bulb, hippocampus, and cerebral cortex), lung, islets, thymus, spleen, lymph nodes, and testis [4, 5]. Kv1.3 channels are also expressed in the inner mitochondrial membrane of normal and cancer cells [4, 6]. In contrast to what is observed for Kv1.3 channels in the plasma membrane, activation of mitochondrial Kv1.3 channels (mito Kv1.3 channels) induces an influx of potassium ions inside the mitochondrial matrix, thereby depolarizing the inner mitochondrial membrane [4, 6]. Activity of Kv1.3 channels plays an important role not only in setting the cell resting membrane potential but also in cell proliferation and apoptosis [4, 7–11]. Activity of Kv1.3 channels is inhibited by many chemically unrelated compounds: heavy-metal cations, small-molecule organic compounds, and venom-isolated oligopeptides [4, 7–10]. The most potent specific inhibitors inhibit the channels at subnanomolar concentrations [9]. Inhibition of Kv1.3 channels by specific inhibitors may be beneficial in therapy of T-lymphocyte-mediated autoimmune diseases (e.g., sclerosis multiplex, type I diabetes mellitus, rheumatoid arthritis, and psoriasis), chronic renal failure, asthma, obesity,

type II diabetes mellitus, cognitive disabilities, and some cancer disorders [4–10]. Recently formulated hypothesis has claimed that the inhibition of T lymphocyte Kv1.3 channels might suppress the "cytokine storm" in severe cases of COVID-19 disease, and this could be a novel therapeutic strategy to combat the disease [12].

Several studies have demonstrated altered expression of Kv1.3 in some cancer types when comparing with normal tissue [4, 13–16]. However, no general pattern of these changes is known at present. The changes depend on the type and the stage of the disease. Cancer tissues may upregulate or downregulate the channels. An increased expression of Kv1.3 channels was observed in the case of breast, colon, smooth muscle (leiomyosarcoma), skeletal muscle (alveolar rabdomyosarcoma), and lymph node cancers and in mature neoplastic B cells in chronic lymphocytic leukemia (B-CLL) [4]. On the other hand, a markedly reduced expression of Kv1.3 channels was detected in a case of breast adenocarcinoma, and there was an inverse correlation between the channel expression and the disease's grade [4]. A significantly reduced expression of the channels was also observed in kidney, bladder, pancreas, lung, brain (astrocytoma, oligodendroglioma, and glioblastoma), stomach, and prostate cancers [4].

Importantly, many different cancer cells lines, which are affected by anticancer activities of statins, express Kv1.3 channels both in the plasma membrane and in mitochondria [1, 4]. Among them are breast cancer cell lines: MCF-7 and MDA-MB-231, colon carcinoma SW-480 and LoVo cells, glioblastoma U87 cell line, leukemic Jurkat T and CEM cells, acute myeloid leukemia (OCI-AML-3) cell line, promyeloytic leukemia HL-60 cell line, pancreatic cancer PANC-1 cell line, and prostate cancer LNCaP cell line [1, 4]. It is known that membrane-permeant small-molecule organic inhibitors of Kv1.3 channels may be able to simultaneously inhibit proliferation of Kv1.3 channel-expressing cancer cells (by the inhibition of plasma membrane Kv1.3 channels) and to induce apoptosis of these cells (by the inhibition of the mito Kv1.3 channels) [4]. The apoptosis of Kv1.3 channel-expressing cancer cells occurred by an activation of the intracellular (mitochondrial) pathway of this process [4]. Importantly, the apoptosis occurred only in the cancer but not in normal cells [4, 17, 18]. Therefore, these inhibitors may potentially be applied in the treatment of some cancer diseases such as, melanoma, pancreatic ductal adenocarcinoma (PDAC), multiple myeloma, and B-type chronic lymphocytic leukemia (B-CLL) [4, 17–20].

In contrast to flavonoids, inhibitory effects of statins on Kv1.3 channels in cancer cells remain unknown. Therefore, in order to elucidate the role of inhibition of these channels in antiproliferative and pro-apoptotic activities of statins on cancer cells, the influence of statins on the activity of Kv1.3 channels in cancer cells needs to be elucidated. Then, the relationship between the putative channel inhibition and anticancer activities of statins must be studied in detail. Since it is known that these activities are augmented upon co-application with flavonoids, effects of co-application of the statins simvastatin and mevastatin and flavonoids on the activity of Kv1.3 channels and the viability of Kv1.3 channel-expressing cancer cells should be elucidated.

2. Influence of statin application on the activity of Kv1.3 channels and viability of Kv1.3 channel-expressing cancer cells

2.1 Influence of statins on the activity of Kv1.3 channels in normal and cancer cells

The first report about the inhibitory effect of statins on Kv1.3 channels appeared in the literature only in 2014 [21]. The authors were looking for immunomodulatory

agents that can be applied as anti-inflammatory drugs. They applied the "whole-cell" patch-clamp technique [22] to study the influence of the statins pravastatin, lovastatin, and simvastatin on the activity of Kv1.3 channels in mice thymocytes. They observed that application of 1 mM of pravastatin significantly reduced the end-of-the-pulse whole-cell Kv1.3 currents, elicited by a sequence of depolarizing voltage steps, probably due to acceleration of the current inactivation rate. This effect was irreversible, since the inactivation rate did not recover to the control value after wash-out of the drug [21].

The authors observed that other tested statins lovastatin and simvastatin exerted much more significant inhibitory effect on the currents at much lower concentrations. Application of both statins at the concentration of 10 μM significantly reduced both the peak and the end-of-the-pulse Kv1.3 currents elicited by a sequence of depolarizing voltage steps. The reduction of the end-of-the-pulse current was more significant than the reduction of the peak current, probably due to acceleration of current inactivation rate upon application of the statins. The inhibitory effect exerted by lovastatin was reversible, because the currents recovered to the control value after wash-out of the drug, but the one exerted by simvastatin was irreversible [21]. Measurements of membrane capacitance showed that application of lovastatin and simvastatin, but not pravastatin, led to a significant decrease of the membrane capacitance. This decrease was abolished after the wash-out of lovastatin, but it remained after the wash-out of simvastatin [21]. The decrease of membrane capacitance was probably due to an increase of membrane thickness upon application of the statins. The increase of membrane thickness may be a consequence of interactions between the statin molecules and the lipid bilayer, leading to perturbations of the bilayer structure. These perturbations were reversible upon application of lovastatin, but irreversible in the case of application of simvastatin. Perturbations of the lipid bilayer structure indirectly reduced the channel activity [21].

A more detailed study on the influence of application of lovastatin on the activity of Kv1.3 channels was performed by Zhao and co-workers [23]. The authors were motivated by a need for the search of immunomodulatory agents to be applied in the treatment of immune-related disorders, especially in T-lymphocyte-mediated autoimmune diseases. The influence of lovastatin on the activity of Kv1.3 channels was studied on the channels expressed both in normal cells – human T lymphocytes isolated from peripheral blood of healthy donors – and in Kv1.3 channel-expressing human lymphoblastic cancer cell line Jurkat, applying the "whole-cell" patch-clamp technique [23]. It was shown that lovastatin inhibited both the peak and the end-of-the-pulse Kv1.3 currents, elicited by a sequence of depolarizing voltage steps, in the concentration-dependent manner. The inhibition of the end-of-the-peak current was more potent than the inhibition of the peak current. This is probably due to a significant acceleration of the current inactivation rate upon application of lovastatin. The inhibitory effect was reversible at all the concentrations between 1 and 100 μM [23]. The half-blocking concentration values (IC_{50}) were equal to 39.81 ± 5.11 μM and 6.92 ± 0.95 μM for the peak and end-of-the-pulse current, respectively [23]. Importantly, the inhibitory effects exerted by lovastatin on the channels expressed in normal and cancer cells were indistinguishable from each other [23]. Therefore, the inhibitory effect of lovastatin on Kv1.3 channels may exert not only the expected immunomodulatory effect but also antiproliferative effect on Kv1.3 channel-expressing cancer cells.

Importantly, it was shown that the inhibitory effect of lovastatin on the channels was significantly diminished when lovastatin was co-applied with internal

tetraethylamine (TEA) or external verapamil [23]. Both of these drugs are inhibitors of Kv1.3 channels [5]. Moreover, the inhibition was significantly weaker in the case of mutant channels, where valine in the position 417 was replaced by alanine (V417A) [23]. This suggests that the channel inhibition is not only a simple consequence of perturbations of the structure of lipid bilayer, but it is also, at least partially, due to the binding of lovastatin molecules to a binding site on the channel protein. This binding site probably overlaps the binding site for internal TEA and external verapamil. Therefore, a co-application of the statin with these drugs leads to a reduction of the lovastatin-induced channel inhibition, due to a competition for the binding site. The inhibition of Kv1.3 channels by lovastatin probably occurs via an "open channel block"- like mechanism [23].

The inhibition of Kv1.3 channels in cancer cells may also occur in the case of application of other statins, which are inhibitors of the channels in normal cells, such as pravastatin and simvastatin [21]. Another possible candidate to be an inhibitor of Kv1.3 channels in cancer cells is mevastatin. This compound is structurally closely related both to lovastatin and to simvastatin, and it shares anticancer activities of simvastatin on Kv1.3 channel-expressing cell line LoVo [3].

A detailed study on the influence of simvastatin, mevastatin, and pravastatin on the activity of Kv1.3 channels in cancer cells was performed in the past years by Teisseyre and co-workers [24]. Since Kv1.3 channels are endogenously and abundantly expressed in human leukemic T cell line Jurkat, these cells were used as a model system of Kv1.3 channel-expressing cancer cells. The study was performed applying the "whole-cell" patch-clamp technique [24].

The authors showed that the application of each one of the statins caused a significant decrease of the peak whole-cell Kv1.3 current recorded upon the application of depolarizing "voltage ramps" (**Figure 1A**, upper panel, [24]). In an agreement with the results obtained earlier by Kazama and co-workers [21], the least potent inhibition of the currents occurred upon the application of pravastatin. Application of this statin at the concentrations up to 50 μM caused a concentration-dependent reduction of the current amplitude to about 0.74 of the initial value. This was accompanied by a remarkable but statistically insignificant acceleration of the current inactivation rate [24]. In contrast to what was observed by Kazama and co-workers [21], the inhibitory effect of pravastatin on the channels was fully reversible, probably because the applied concentrations were much lower (50 μM was a maximum) [24].

A much more significant inhibition of the channel activity occurred upon the application of both simvastatin and mevastatin [24]. Application of simvastatin caused a concentration-dependent reduction of the peak "ramp current" to about 0.28 of the control value (**Figure 1A**, lower panel). The value of the IC_{50} parameter was equal to 4.85 ± 0.011 μM [24]. The current did not recover after "wash-out" of the statin (**Figure 1A**, lower panel). This is in accordance with what was observed by Kazama and co-workers, who showed that the inhibitory effect of simvastatin was partially irreversible [21].

The reduction of the current amplitude was accompanied by a significant acceleration of the current inactivation (**Figure 1B**, lower panel), which was revealed by a large reduction of the value of inactivation time constant, calculated for the currents recorded upon the application of depolarizing voltage steps (**Figure 1B**, upper panel) [24]. On the other hand, no significant change of the current activation rate was observed (**Figure 1B**, lower panel, [24]).

The inactivation phase of the currents did not recover completely after the wash-out of the drug (**Figure 1B**, lower panel) [24]. This indicates that the acceleration of

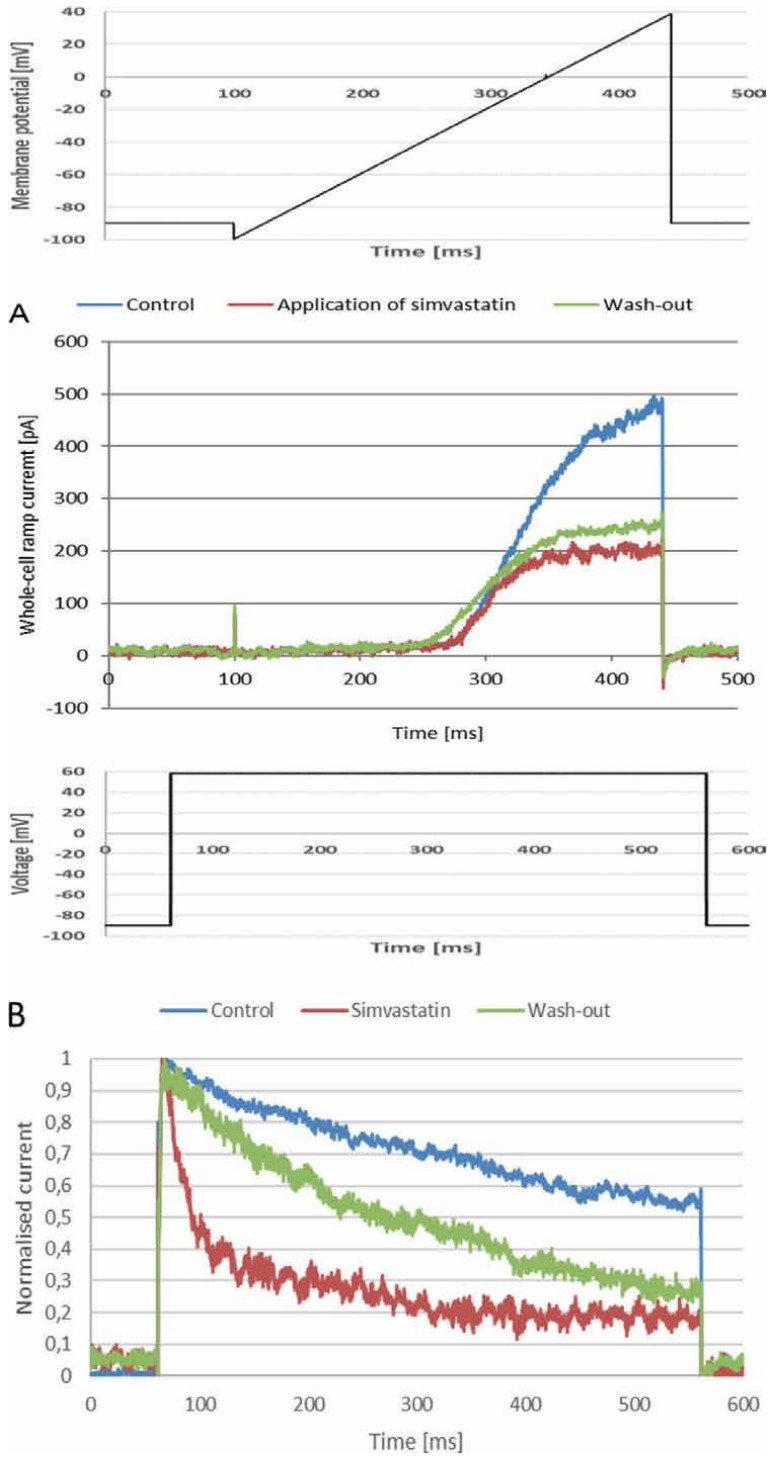

Figure 1.
(A) Whole-cell Kv1.3 ramp currents (lower panel) recorded in a Jurkat T cell upon an application of the depolarizing "voltage ramp" (upper panel), (B) normalized whole-cell Kv1.3 currents (lower panel) recorded on the same cell upon an application of a depolarizing voltage step (upper panel). The concentration of simvastatin was equal to 30 μM.

inactivation was partially irreversible. Such a partial irreversibility was observed at all the concentrations applied [24]. This is in accordance with what was observed earlier by Kazama and co-workers [21].

Application of mevastatin caused a concentration-dependent reduction of the peak "ramp current" to about 0.32 of the control value at the concentration of 30 μM (**Figure 2A**) [24].

The value of the IC_{50} parameter was equal to 6.04 ± 0.4 μM [24]. Thus, the inhibitory effect exerted on the channels by mevastatin was comparable to the one exerted by simvastatin. However, in contrast to what was observed for simvastatin, the inhibitory effect of mevastatin was fully reversible at all applied concentrations (**Figure 2A**) [24].

The reduction of the current amplitude was accompanied by a significant acceleration of the current inactivation, which was revealed by a large reduction of the value of inactivation time constant, calculated for the currents recorded upon the application of depolarizing voltage steps (**Figure 2B**) [24]. On the other hand, no significant change of the current activation rate was observed (**Figure 2B**). However, in contrast to what was observed for simvastatin, the inactivation phase of the currents recovered completely after the wash-out of mevastatin (**Figure 2B**) [24]. Thus, the acceleration of inactivation was fully reversible, and such a reversibility was observed at all applied concentrations [24].

The inhibitory effects exerted on Kv1.3 channels in Jurkat T cells by simvastatin and mevastatin resemble the effect exerted by lovastatin [23]. Also, in the case of application of lovastatin, the channel inhibition is accompanied by a significant acceleration of the whole-cell Kv1.3 currents, without a significant change of the activation rate [23]. The mechanism of Kv1.3 channel inhibition by the statins is probably complex and includes both specific interactions of the statin molecules with a binding site on the channel protein in an "open channel block"–like mechanism and nonspecific interactions with the lipid bilayer, leading to perturbations in the bilayer's structure, which, in turn, affect the channel activity. Both specific and nonspecific interactions finally lead to a stabilization of the channel proteins in an inactivated (nonconducting) state [24]. Since pravastatin is much less lipophilic than the others, the mechanism of its inhibitory effect is probably different, and it may be related to the interactions of the statin molecules with the external vestibule of the channel [24].

Finally, Wang and co-workers showed that application of simvastatin significantly inhibited mRNA and protein expression of Kv1.3 channels in human monocytic leukemia THP-1 cells [25]. Electrophysiological recordings performed applying the "whole-cell" patch-clamp technique showed that application of simvastatin in the range of 1–100 μM reduced the peak whole-cell Kv1.3 current recorded upon application of a sequence of depolarizing voltage steps [25]. The value of the IC_{50} parameter was equal to 8.75 ± 1.25 μM [25]. This value was significantly higher than the value calculated by Teisseyre and co-workers [24]. Moreover, in contrast to what was observed by other authors [21, 24], application of simvastatin in monocytes did not accelerate the inactivation of the recorded "whole-cell" potassium currents [25]. This is probably due to the fact that the recorded currents are apparently and significantly contaminated by slowly activating and non-inactivating voltage-gated potassium currents, which are resistant both to simvastatin and to a specific Kv1.3 channel inhibitor, margatoxin (MgTX) [25]. These currents, which may be due to an activation of an unknown type of voltage-gated potassium channel, may mask the effect of simvastatin on the inactivation kinetics of Kv1.3 currents.

The influence of the statins pravastatin, lovastatin, simvastatin, and mevastatin on the activity of Kv1.3 channels is summarized in **Table 1**.

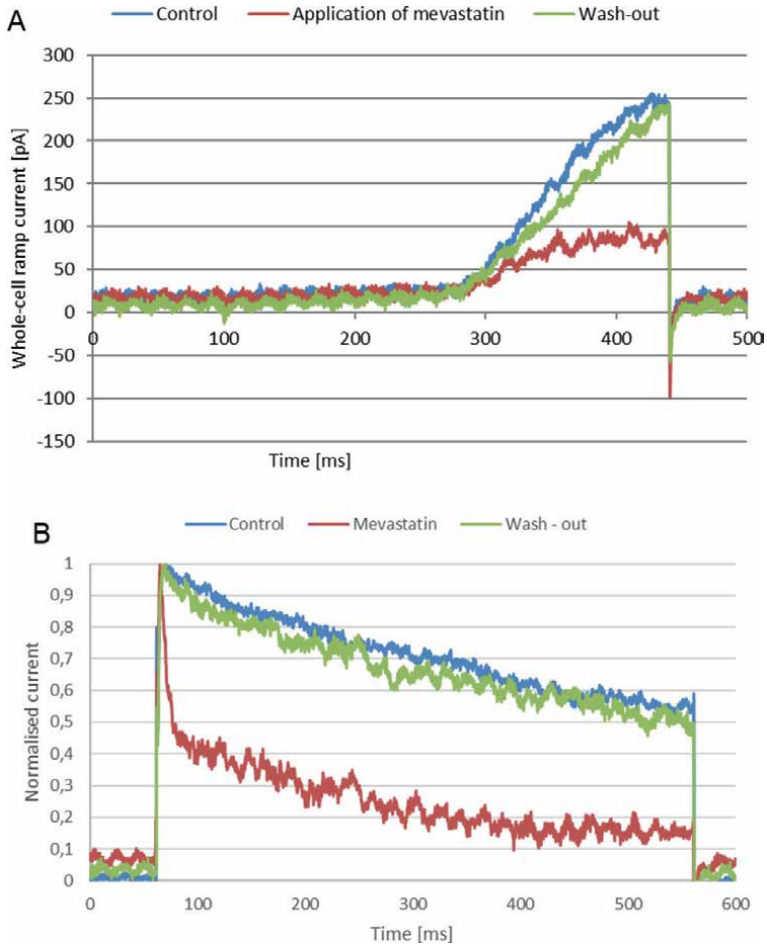

Figure 2.
(A) Whole-cell Kv1.3 ramp currents, (B) normalized whole-cell Kv1.3 currents recorded on a Jurkat T cell applying experimental protocols same as in **Figure 1***. The concentration of mevastatin was equal to 30 μM.*

2.2 Anti-proliferative and pro-apoptotic activities of the statins on Kv1.3 channel-expressing cancer cells

The mechanism of antiproliferative effect of statins on Kv1.3 channel-expressing cancer cells was studied in detail in the case of application of lovastatin on human leukemic T-cell line Jurkat, which can be considered as a model system of Kv1.3 channel-expressing cancer cells [23]. It was shown that the application of lovastatin caused a concentration-dependent decrease of influx of calcium ions through calcium release-activated channels (CRAC) [23]. This, in turn, caused a concentration-dependent down-regulation of calcium-related transcription factors: NFAT1 (nuclear factor of activated T cells) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) [23]. Finally, the application of lovastatin caused a concentration-dependent inhibition of production of T-cell growth factor Interleukin-2 (IL-2) and cell proliferation [23]. The antiproliferative effect of lovastatin was partially but not completely abrogated when Kv1.3 channels were knocked down by applying the Kv1.3 channel-specific Kv1.3 small-interfering RNA (Kv1.3-siRNA) [23]. This indicates that

Name of the statin	Inhibitory effect	Maximal inhibition	References
Pravastatin	Dose-dependent reduction of the "whole-cell" current combined with acceleration of inactivation. Partially irreversible at the concentration of 1 mM	Ca. 36% at the concentration of 1 mM	[21, 24]
Lovastatin	Dose-dependent reduction of the "whole-cell" current combined with acceleration of inactivation. Reversible	Ca. 90% at the concentration of 100 μM	[21, 23]
Simvastatin	Dose-dependent reduction of the "whole-cell" current combined with acceleration of inactivation. Partially irreversible	Ca. 80% at the concentration of 100 μM	[21, 24, 25]
Mevastatin	Dose-dependent reduction of the "whole-cell" current combined with acceleration of inactivation. Reversible	Ca. 68% at the concentration of 30 μM	[24]

Table 1.
Summary of inhibitory effects of the statins on Kv1.3 channels.

the antiproliferative effect of lovastatin on Kv1.3 channel-expressing cancer cells is complex, and it includes both Kv1.3 channel-dependent and channel-independent pathways [23].

The Kv1.3 channel-dependent antiproliferative effect of lovastatin on Jurkat T cells is typical for Kv1.3 channel inhibitor-mediated inhibition of proliferation of normal and cancer cells expressing these channels. According to the theoretical "membrane potential model," inhibition of Kv1.3 channels leads to depolarization of the cell membrane and reduction of the electrochemical "driving force" for entry of calcium ions through the calcium CRAC channels (**Figure 3**) [4]. Reduction of calcium ion influx inhibits all the downstream calcium-dependent processes, including activation of transcription factors, Interleukin-2 production, and, finally, the cell proliferation [4]. In such a case, the cell cycle is halted at the checkpoint between the G1 and the S phase [4, 5, 8–10].

Lovastatin exerts antiproliferative effect on many other cancer cell lines expressing Kv1.3 channels. Among them are breast cancer MCF-7 and MDA-MB-231 cell lines; colon carcinoma HCT116, SW480, and LoVo cells; glioblastoma U87 cell line; leukemic CEM and OCI-AML 3 cell lines; promyelotic leukemia HL60 cell line; pancreatic cancer PANC-1 cells; and prostate cancer LNCaP cells [1]. Interestingly, the antiproliferative effect of lovastatin on Kv1.3 channel-expressing cancer cells is shared by other statins, which are inhibitors of Kv1.3 channels in these cells. Pravastatin inhibits proliferation of leukemic Jurkat T and CEM cells, whereas simvastatin, in addition to the leukemic cell lines mentioned above, also inhibits proliferation of breast cancer MCF-7 and MDA-MB-231 cell lines [1]. Moreover, simvastatin and mevastatin inhibit proliferation of colon carcinoma LoVo cells and their doxorubicin-resistant subline LoVo/DX [3]. Whether these antiproliferative effects are related to inhibition of Kv1.3 channels remains to be elucidated.

It is known that lipophilic small-molecule organic inhibitors of Kv1.3 channels may induce apoptosis of Kv1.3 channel-expressing cancer cells by an inhibition of mito Kv1.3 channels [4]. The apoptosis occurs by activation of its intracellular (mitochondrial) pathway. Inhibitors of mito Kv1.3 channels mimic the action of the pro-apoptotic protein Bax. The inhibition of mito Kv1.3 channels facilitates the production

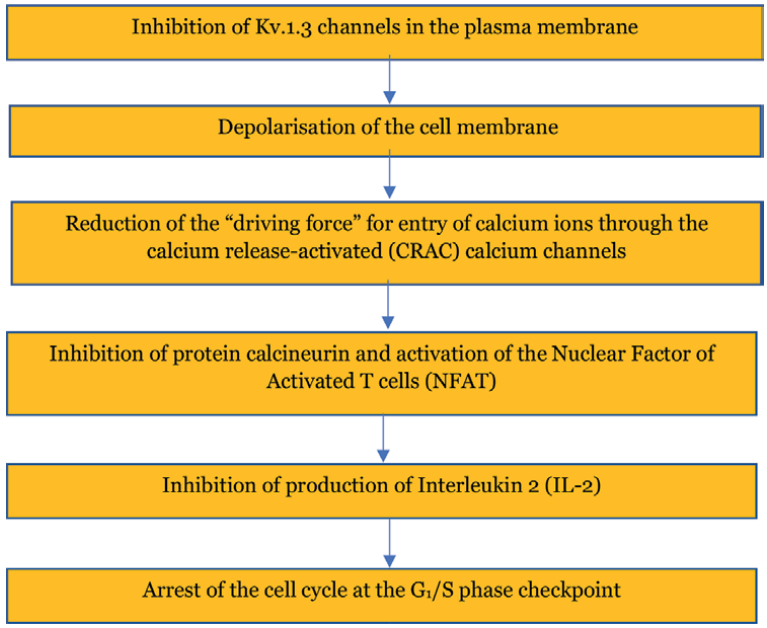

Figure 3.
Scheme of events upon inhibition of proliferation of Kv1.3 channel-expressing cells by the channel inhibitors according to the "membrane potential model."

of Reactive Oxygen Species (ROS) by mitochondria. This, in turn, promotes opening of mitochondrial Permeability Transition Pores (PTP), loss of mitochondrial membrane potential (MMP), release of mitochondrial cytochrome c, and activation of pro-apoptotic enzymes, caspase-9 and caspase-3 (**Figure 4**) [4].

Importantly, as it was mentioned previously, induction of cancer cell apoptosis by inhibitors of mito Kv1.3 channels may occur only in Kv1.3 channel-expressing cancer cells, whereas normal cells expressing these channels are saved [4]. This is because of a combination of an elevated expression of Kv1.3 channels in some cancer cells and elevated basal ROS production in these cells [4]. Such a combination does not occur in normal cells, even in those that overexpress Kv1.3 channels [4].

It was shown that the application of simvastatin and mevastatin induced apoptosis of Kv1.3 channel-expressing cancer cell line LoVo and its doxorubicin-resistant subline LoVo/Dx [3]. Application of the statins induced activity of caspase-3 and fragmentation of DNA in the above-mentioned cell lines [3]. Results published recently by Teisseyre and co-workers [26] showed that the application of both simvastatin and mevastatin induced apoptosis of Jurkat T cells. The pro-apoptotic activity was stronger in case of mevastatin than upon the application of simvastatin. The concentration required to reduce viability of the cells to 50% of the control value (IC_{50}) was equal to 22.5 μM in case of mevastatin application. In case of application of simvastatin, the viability was reduced to about 60% even upon application of 40 μM of the statin. The increase of activity of caspase-3 and elevation of cleaved caspse-3 in these cells upon application of the statins were accompanied by a loss of the mitochondrial membrane potential (MMP) [26]. This may indicate that the statin-induced apoptosis of Jurkat T cells is a result of the activation of the mitochondrial pathway of this process. The apoptosis may be triggered by inhibition of mito Kv1.3 channels by the statins (**Figure 4**).

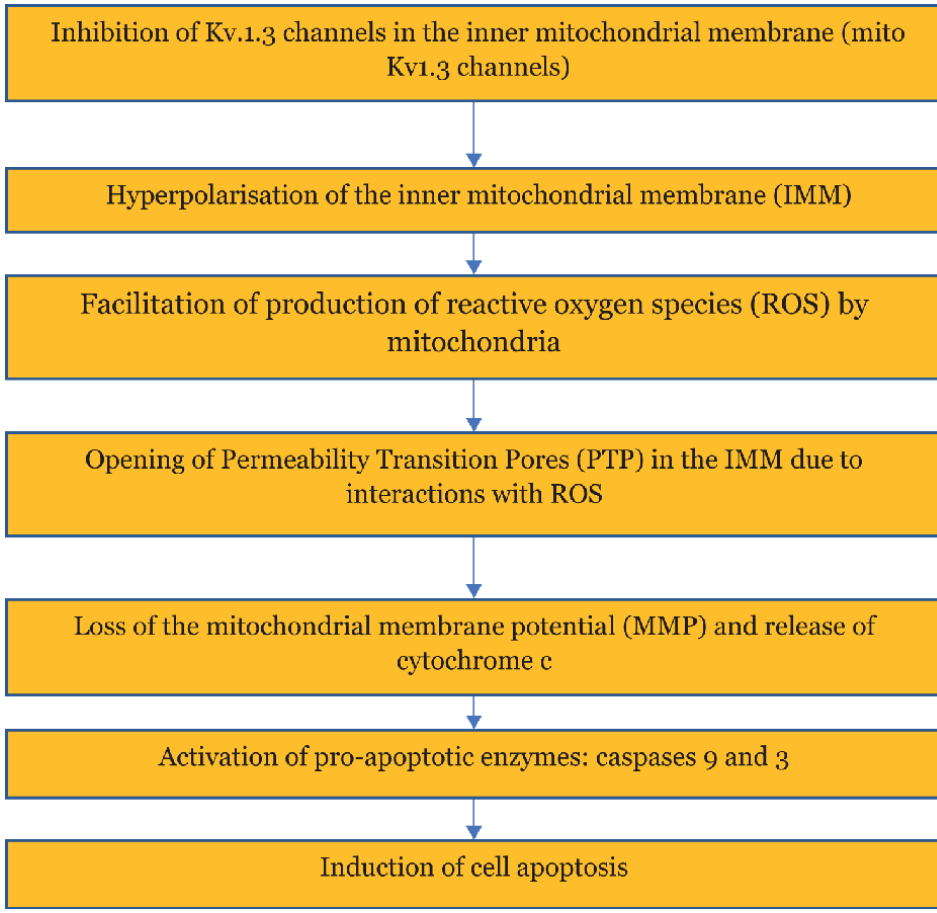

Figure 4.
Scheme of events upon induction of apoptosis of Kv1.3 channel-expressing cancer cells by the inhibitors of mito Kv1.3 channels.

The pro-apoptotic activity of simvastatin and mevastatin on Kv1.3 channel-expressing cancer cells is shared by another statin, which is an inhibitor of Kv1.3 channels in cancer cells, lovastatin [1]. It was shown that the application of lovastatin induced apoptosis of Kv1.3 channel-expressing colon carcinoma HCT116, SW480, and LoVo cell lines; human leukemic Jurkat T and CEM cells; promyelocytic leukemia HL60 cells; and prostate cancer LNCaP cell line [1]. Moreover, the application of pravastatin induced apoptosis of human leukemic Jurkat T and CEM cells [1].

A short list of Kv1.3 channel-expressing cancer cells, on which statin inhibitors of the channels exert antiproliferative and anti-apoptotic effects, is given in **Table 2**.

Whether these pro-apoptotic activities are related to inhibition of mito Kv1.3 channels remains to be elucidated.

2.3 Influence of co-application of statins and flavonoids on the activity of Kv1.3 channels and viability of Kv1.3 channel-expressing cancer cells

Effects of co-application of the statins simvastatin and mevastatin and the flavonoids 8-prenylnaringenin (8-PR), 6-prenylnaringenin (6-PR), acacetin (Ac),

Name of the statin	Antiproliferative and pro-apoptotic effects on Kv1.3 channel-expressing cancer cells	References
Pravastatin	Human leukemic Jurkat T and CEM	[1]
Lovastatin	Human leukemic Jurkat T, CEM, and OCI-AML 3; breast cancer MCF-7 and MDA-MB-231; colon carcinoma HCT116, SW480, and LoVo; glioblastoma U87; promyelotic leukemia HL60; pancreatic cancer PANC-1; prostate cancer LNCaP	[1, 23]
Simvastatin	Human leukemic Jurkat T, CEM; breast cancer MCF-7 and MDA-MB-231; colon carcinoma LoVo and LoVo/DX	[1, 3, 26]
Mevastatin	Human leukemic Jurkat T; colon carcinoma LoVo and LoVo/DX	[3, 26]

Table 2.
List of Kv1.3 channel-expressing cancer cells, on which the statins exert antiproliferative and pro-apoptotic effects.

chrysin (Ch), and a chalcone xanthohumol (X) on the activity of Kv1.3 channels and the viability of Kv1.3 channel-expressing human leukemic T cell line Jurkat were studied in detail recently [26]. Each of these compounds is an effective inhibitor of Kv1.3 channels in Jurkat T cells [4, 24]. Moreover, the application of 6-PR, Ac, and Ch significantly reduced the viability of Jurkat T cells [27]. It is shown that co-application of mevastatin with all the flavonoids and xanthohumol significantly augmented the inhibitory effect of mevastatin on Kv1.3 channels in Jurkat T cells [26]. Moreover, a significant augmentation of the inhibitory effect exerted on the channels by simvastatin was observed upon a co-application of this statin with 8-PR, 6-PR, and Ch [26].

In most cases, the inhibitory effect exerted on Kv1.3 channels upon co-application of the statins with the flavonoids may be considered as additive [26]. In such a case, the relative peak current recorded upon co-application of the statins with the flavonoids is equal to the product of multiplication of the relative currents recorded upon application of each compound alone. **Table 3** shows comparisons of relative peak currents recorded upon co-application of the statins with the flavonoids with theoretical values calculated applying the additive inhibition model [26].

As it is shown in **Table 3**, the theoretical and experimental values of the relative peak currents are comparable to each other in case of co-application of simvastatin with Ch and upon co-application of mevastatin with all the compounds except for 8-PR. In these cases, the inhibitory effects of the statins and flavonoids can be considered as additive. However, in case of co-application of both statins with 8-PR and simvastatin with 6-PR, the experimental values of relative peak currents are significantly lower than the theoretical ones (**Table 3**). In such cases, the inhibitory effects of the statins co-applied with flavonoids are rather synergistic than additive [26]. On the other hand, in case of co-application of simvastatin with X and Ac, the experimental values of relative peak currents are significantly higher than predicted by the theoretical model (**Table 3**). In these cases, the inhibitory effect exerted on the channels upon co-application of the compounds is comparable to the effect exerted by simvastatin applied alone; thus, no additivity is observed [26].

Additive or synergistic inhibitory effects exerted on Kv1.3 channels upon co-application of statins with flavonoids may be related to augmented anti proliferative and pro-apoptotic effects exerted by these compounds on Kv1.3 channel-expressing cancer cells.

Recently published data have shown that co-application of the statins simvastatin and mevastatin with the flavonoids, except for Ac, and xanthohumol leads to a

Simvastatin co-applied with:	**8-PR**	**6-PR**	**X**	**Ac**	**Ch**
Theoretical values	0.195	0.395	0.36	0.32	0.285
Experimental values	0.12	0.26	0.50	0.53	0.25
Inhibitory effect upon co-application	Synergistic	Synergistic	Not additive	Not additive	Additive
Mevastatin co-applied with:	8-PR	6-PR	X	Ac	Ch
Theoretical values	0.16	0.32	0.31	0.26	0.23
Experimental values	0.09	0.29	0.30	0.30	0.26
Inhibitory effect upon co-application	Synergistic	Additive	Additive	Additive	Additive

Table 3.
Comparison of theoretical and experimental values of relative peak Kv1.3 currents recorded upon co-application of the statins with flavonoids and xanthohumol.

significant reduction of viability of Jurkat T cells, revealed by a decrease of the IC_{50} value [26]. The values of IC_{50} upon application of the flavonoids and xanthohumol alone and in a combination with the statins are depicted in **Table 4**.

The most significant reduction of this value occurred upon co-application of simvastatin with Ch and mevastatin with 8-PR, Ch, and X (**Table 4**). More significant reduction occurred upon co-application of mevastatin with the flavonoids than when simvastatin was co-applied with these compounds (**Table 4**). This may be due to the fact that mevastatin exerted stronger pro-apoptotic effect on Jurkat T cells than simvastatin [26]. Interestingly, in all these cases, there was an additive or synergistic inhibitory effect of the compounds on Kv1.3 channels (**Table 3**). On the other hand, no significant change of the IC_{50} value was observed upon co-application of simvastatin with X (**Table 4**). This may be related to a lack of additive inhibitory effects on Kv1.3 channels upon co-application of these compounds (**Table 3**).

It was also shown that co-application of simvastatin with the flavonoids, except for Ac, led to a significant increase of the activity of caspase-3 [26]. Even more significant increase of activity of this enzyme was observed upon co-application of mevastatin with the flavonoids, except for Ac, and X [26]. Moreover, it was shown that the expression of an active (cleaved) form of caspase-3 was significantly higher when Jurkat T cells were treated with mevastatin in combination with Ch, X, and 8-PR [26]. These results may indicate that the reduction of viability of Jurkat T cells upon treatment of statins applied alone and in combination with flavonoids is due to the induction of apoptosis of these cells.

Flavonoid	**Applied alone**	**Co-applied with simvastatin**	**Co-applied with mevastatin**
8-PR	> 40 μM	26.9 μM	7.1 μM
6-PR	> 40 μM	38.9 μM	34.8 μM
Ch	26.2 μM	10.8 μM	8.3 μM
X	32.5 μM	30.8 μM	3.8 μM

Table 4.
Comparison of the IC_{50} value of decrease of viability of Jurkat T cells upon application of the flavonoids and xanthohumol alone and in combination with the statins simvastatin and mevastatin.

Finally, it was shown that co-application of the statins with the flavonoids and xanthohumol increased the percentage of Jurkat T cells with depolarized mitochondria. It is known that the loss of mitochondrial membrane potential (MMP) is one of hallmarks of induction of the mitochondrial pathway of apoptosis [4]. Thus, the increased percentage of depolarized mitochondria may indicate that Jurkat T cells treated with the statins and the statins co-applied with the flavonoids and xanthohumol undergo apoptosis that mainly occurs by activation of its intracellular (mitochondrial) pathway. The loss of MMP may be a consequence of the inhibition of mito Kv1.3 channels by lipophilic inhibitors of the channels [4]. A significant increase of percentage of the cells with depolarized mitochondria was observed when mevastatin was co-applied with all the flavonoids (including Ac) and X [26]. A significant increase of this parameter was also observed upon co-application of simvastatin with Ac and Ch. Upon co-application of the statin with 8-PR and 6-PR, the percentage of Jurkat T cells with depolarized mitochondria increased less significantly but still remarkably [26]. This may indicate that the apoptosis of Jurkat T cells upon co-application of the statins with the flavonoids occurs as a result of activation of the mitochondrial pathway of this process. The activation of apoptosis may be a result of inhibition of mito Kv1.3 channels by the mixture of compounds, such as it may occur upon the application of the statins alone (**Figure 4**) [26].

3. Possible further directions

As it was mentioned above, more studies have to be done to further elucidate the role of inhibition of Kv1.3 channels in anti-cancer activities of statins. First of all, more statins should be tested from the point of view of their ability to inhibit Kv1.3 channels. It is known that antiproliferative and pro-apoptotic effects on Kv1.3 channel-expressing cancer cells are shared by other statins, such as cerivastatin, fluvastatin, and atorvastatin [1]. However, influence of these statins on the activity of Kv1.3 channels still remains unknown.

Secondly, studies on antiproliferative and pro-apoptotic effects of statin inhibitors of Kv1.3 channels should be extended to Kv1.3 channel-expressing cancer cells other than Jurkat T cells. It is known that lipophilic inhibitors of Kv1.3 channels share the ability to selectively induce apoptosis of Kv1.3 channel-expressing cell lines representing various types of cancer disorders [4]. Good candidates may be human neoplastic B-CLL cells, human osteosarcoma SAOS-2 cell line, mouse melanoma B16F10 cells, pancreatic ductal adenocarcinoma (PDAC) cell lines (i.e., As PC-1, Capan-1, Panc-1, Mia PaCa 2, Bx PC-3, Colo357), and glioblastoma GL261, A172, and LN308 cells [4].

Finally, effects of co-application of other statins (e.g., cerivastatin, fluvastatin, atorvastatin) with flavonoids on the activity of Kv1.3 channels and viability of Kv1.3 channel-expressing cancer cells should be studied in detail.

4. Conclusions

In this chapter, it was shown that the statins lovastatin, mevastatin, pravastatin, and simvastatin were all inhibitors of Kv1.3 channels in normal and cancer cells. The ability to inhibit Kv1.3 channels may be shared by other statins, which exert

antiproliferative and pro-apoptotic effects on Kv1.3 channel-expressing cancer cells, such as cerivastatin, fluvastatin, and atorvastatin. In order to elucidate the putative inhibitory effects of these statins on the channels, complex electrophysiological studies will have to be carried out.

It is also known that the inhibition of Kv1.3 channels may be related to antiproliferative and pro-apoptotic effects of the statins on Kv1.3 channel-expressing cancer cells. The putative mechanism was studied in a model system—human leukemic T cell line Jurkat. The mechanisms of these activities are probably complex and include both the Kv1.3 channel-dependent and the channel-independent pathways. Studies on antiproliferative and pro-apoptotic effects of the statins on other Kv1.3 channel-expressing cancer cells, such as human neoplastic B-CLL cells, human osteosarcoma SAOS-2 cell line, mouse melanoma B16F10 cells, pancreatic ductal adenocarcinoma (PDAC) cell, and glioblastoma cell lines, remain to be performed.

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Conflict of interest

The authors declare they have no conflict of interest.

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Chapter 7

A Review of Statins and COVID-19

Justin H. Shiu, Heather N. Pham, Navneet Singh and Alexander J. Sweidan

Abstract

Statins are a well-established class of β-Hydroxy β-methylglutaryl Coenzyme A (HMG-CoA) reductase inhibitors that have recently been discussed as a possible therapeutic in COVID-19. The breadth of this chapter reviews the evidence for use of statins alone or in combination with other drugs as treatment for patients hospitalized with moderate to severe COVID-19. Discussion will include a (1) biochemical argument for the role of statins in COVID-19, (2) a systematic literature review of relevant studies to date, and (3) an investigation into early-phase interventional studies. Outcome measures based on all aforementioned relevant studies will be clearly defined and compared.

Keywords: COVID-19, SARS-CoV-2, coronavirus disease, statins, statin therapy

1. Introduction

The COVID-19 pandemic that emerged from the novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) will be remembered as one of the worst outbreaks in modern history. To date, over 651 million individuals worldwide have tested positive for the disease with over 6 million confirmed deaths [1]. Research from the scientific and medical communities has primarily focused on disease management, specifically in hospitalized patients who have a considerable risk of progression to multi organ failure, acute respiratory distress syndrome, and death. These efforts have included evaluating the effectiveness of various antiviral and anti-inflammatory drugs. Antiviral therapies, such as remdesivir, molnupiravir, and nirmatrelvir with ritonavir; immunomodulators, such as corticosteroids, baricitinib, and tocilizumab; and monoclonal antibodies, such as casirivimab with imdevimab, sotrovimab, and bamlanivimab with etevimab are all authorized therapeutics by the World Health Organization (WHO), National Institutes of Health (NIH), and the U.S. Food and Drug Administration (FDA) for the treatment of COVID-19 [2–4]. While these interventions have proven successful in treating the disease, the systemic adverse effects from a variety of these immunosuppressive agents leaves much to be desired [5]. A number of observational studies and clinical trials have been conducted worldwide to assess the role of repurposing existing medications to increase survival rates and lower morbidity in COVID-related hospitalizations [6–10].

Statins are a class of oral β-Hydroxy β-methylglutaryl Coenzyme A (HMG-CoA) reductase inhibitors that was first approved for commercial use in the United States in

1987 for the primary and secondary prevention of atherosclerotic cardiovascular disease [11]. HMG-CoA reductase is a rate-controlling enzyme that catalyzes the conversion of HMG-CoA to mevalonate in the biochemical pathway of cholesterol metabolism [12–16]. Inhibition of this reductase has been well-associated with the reduction of overall serum cholesterol and improvement of lipid profile in humans [17]. In recent years, other studies have noted a variety of beneficial, "pleiotropic" immunomodulatory effects that reduce inflammation by downregulating expression of inflammatory cytokines, inhibiting thrombogenic response, and reducing oxidative stress [18–22].

The role of statins has been previously studied in other viral respiratory infections. Similar to COVID-19, influenza viruses have been known to induce a cytokine storm, leading to ARDS [23]. A recently completed clinical trial by [24] in 2019 demonstrated that patients who were administered atorvastatin 40 mg orally daily for 5–7 days showed a significant improvement in influenza-related outcomes when compared to the placebo group. This conclusion was also supported by multiple large observational studies done by Vandermeer et al. [25] and Mortensen et al. [26]. In the setting of respiratory syncytial virus (RSV) infection, a study by Malhi et al. [27] to assess a novel approach to screening for RSV inhibitors provides strong evidence that modulation of lipid metabolites by statins decreases production of viral particles in-vitro. Notably, RSV and many other viruses including influenza A, rhinoviruses, and adenoviruses have all been observed to favor lipid-rich environments for infection and upregulate lipid metabolic enzymes such as HMG-CoA reductase [28–32].

Statin therapy mediates proinflammatory pathways by inhibiting SARS-CoV-2 replication, suppressing the release of inflammatory factors, and attenuating cytokine storms [33–35]. Upon initial exposure to lung tissue, SARS-CoV-2 binds to angiotensin-converting-enzyme 2 (ACE2) receptors to replicate and infect other ACE2 tissue cells [36]. The suppression of cholesterol production by HMG-CoA reductase inhibitors causes the disruption of lipid raft formation, negatively affecting the viral replication process [37, 38]. Secondly, SARS-CoV-2 tends to bind to toll-like-receptors (TLR) leading to the overexpression of the MYD88 gene, which induces activation of nuclear factor kappa B (NF-kB), a master regulator of proinflammatory gene expression [39, 40]. In rat models, statins are reportedly able to inhibit NF-kB and preserve MYD88 levels after a proinflammatory trigger [34]. Thirdly, SARS-CoV-2 is noted to uncontrollably increase production of inflammatory cytokines including Interleukin (IL) 6, IL-2, IL-7, IL-10, etc. leading to a higher risk of development of a cytokine storm [41, 42]. In experimental studies, statins were shown to attenuate pulmonary inflammation by suppressing the production of such cytokines [35]. The combined pleiotropic effects of statin therapy makes it a promising candidate to mitigate respiratory injury from the SARS-CoV-2 virus.

The breadth of this chapter reviews existing and prospective observational and interventional studies repurposing statins for treatment of COVID-19.

2. Search methodology

This literature search identified studies that evaluate antecedent and/or subsequent statin therapy during COVID-related hospitalization. The search included observational studies, randomized control trials, and prospective/existing clinical trials that assess the efficacy of statin therapy on COVID-19 outcomes. Excluded were meta-analyses, review articles, case reports, case series, book chapters, author responses, and news articles.

The WHO COVID-19 Database, which contains global literature on COVID-19 from several major online databases, was utilized in conjunction with PubMed, ClinicalTrials.gov, and NIH Clinical Center. Keywords searched for within the Title and Abstract consisted of *SARS-CoV-2, COVID-19, coronavirus disease, statins, statin therapy, atorvastatin, fluvastatin, lovastatin, pravastatin, pitavastatin, rosuvastatin, simvastatin.* Additional manual searches were performed within the bibliography of chosen articles to include other relevant titles.

Quality assessment of observational studies was performed using the Newcastle-Ottawa Scale (NOS), while the risk of bias of randomized controlled trials was determined using the Cochrane tool for randomized trials (RoB 2.0) [43, 44]. In the former, articles were independently screened and rated by two authors based on NOS criteria. Scores were subsequently compared and any discrepancies were discussed and resolved. Studies with a low risk of bias using RoB 2.0 and a "High" NOS rating were given priority.

3. Evidence

Herein, we describe 26 appropriately screened studies that provide evidence regarding the role of statins in the treatment of COVID-19. Of the aforementioned total, 16 constitute observational studies (see **Table 1**). Four are completed interventional clinical trials (see **Table 2**). Six account for active, ongoing clinical trials (see **Table 3**).

3.1 Observational studies

In a retrospective cohort study conducted in India, Umakanthan et al. [45] compared the clinical outcome and laboratory results of prior statin use (n = 524) to non-statin use (n = 1102) of patients hospitalized with COVID-19. A 1:1 propensity-score matching (PSM) was also performed with 384 statin users and 384 non-statin users. Laboratory results revealed that statin users had a lower mean white blood cell count ($P < 0.01$), C-reactive protein (CRP) ($P < 0.001$), and more favorable total lipid profiles when compared to non-statin COVID-19 patients. Additionally, they found statin use was associated with lower odds of mortality ($P < 0.001$). There were no significant differences in mechanical ventilation ($P = 0.07$) and hemodialysis ($P = 0.41$) between antecedent statin users and non-statin users.

Zhang et al. compared the in-hospital use of statin (n = 1219) and non-statin treatment (n = 12,762) in a retrospective cohort study on 13,981 COVID-19 patients over 21 hospitals in Hubei Province, China [46]. A mixed-effect Cox model after PSM revealed the risk for 28-day all-cause mortality in the statin group versus non-statin group to be 5.2% and 9.4%, respectively, with an adjusted hazard ratio (HR) of 0.58 ($P = 0.001$). Statin use was also associated with lower risk of mortality based on the mixed-effect Cox model ($P = 0.001$) and marginal structural model analysis ($P = 0.032$). Additionally, lower incidence of mechanical ventilation ($P < 0.001$), ICU admission ($P = 0.001$), and development of ARDS ($P = 0.015$) were associated with statin treatment via Cox model analysis. Furthermore, 319 participants of the 1219 statin users with hypertension were treated with an additional angiotensin-converting enzyme inhibitors (ACEi)/angiotensin II receptor blockers (ARBs) during hospitalization; however, co-treatment was not associated with any significant benefit in the cohort through analysis using a Cox model ($P = 0.074$), mixed-effect Cox model ($P = 0.018$), or marginal structure model ($P = 0.576$).

Observational studies					
#	**Authors**	**Title**	**Location**	**Study Design**	**NOS Assessment**
1	Umakanthan, et al.	The effect of statins on clinical outcome among hospitalized patients with COVID-19: A multi-centric cohort study	India	retrospective cohort	High
2	Zhang et al.	In-hospital use of statins is associated with a reduced risk of mortality among individuals with COVID-19	Hubei Province, China	retrospective cohort	High
3	Tan et al.	Statin use is associated with lower disease severity in COVID-19 infection	Singapore	retrospective cohort	Fair
4	Vahedian-Azimi et al.	Association of in-hospital use of statins, aspirin, and renin-angiotensin-aldosterone inhibitors with mortality and ICU admission due to COVID-19	Iran	retrospective cohort	Fair
5	Rodriguez-Nava et al.	Atorvastatin associated with decreased hazard for death in COVID-19 patients admitted to an ICU: a retrospective cohort study	Illinois, United States	retrospective cohort	Fair
6	Spiegeleer et al.	The effects of ARBs, ACEis, and statins on clinical outcomes of COVID-19 infection among nursing home residents	Belgium	retrospective cohort	High
7	Oh et al.	Statin therapy and the risk of COVID-19: A cohort study of the National Health Insurance service in South Korea	South Korea	retrospective cohort	High
8	Cariou et al.	Routine use of statins and increased COVID-19 related mortality in inpatients with type 2 diabetes: Results from the CORONADO study	France	retrospective cohort	High
9	Fan et al.	Association of statin use with the in-hospital outcomes of 2019-coronavirus disease patients: A retrospective study	Wuhan, China	retrospective cohort	High
10	Peymani et al.	Statins in patients with COVID-19: a retrospective cohort study in Iranian COVID-19 patients	Shiraz Province, Iran	retrospective cohort	High

Observational studies					
#	**Authors**	**Title**	**Location**	**Study Design**	**NOS Assessment**
11	Israel et al.	Identification of drugs associated with reduced severity of COVID-19 – a case–control study in a large population	Israel	case control	High
12	Ayeh et al.	Statins use and COVID-19 outcomes in hospitalized patients	Maryland, United States	retrospective cohort	High
13	Bifulco et al.	The benefit of statins in SARS-CoV-2 patients: further metabolic and prospective clinical studies are needed	Mlan, Italy	retrospective cohort	Fair
14	Maric et al.	Decreased mortality rate among COVID-19 patients prescribed statins: Data from electronic health records in the US	United States	retrospective cohort	High
15	Saeed et al.	Statin Use and in-hospital mortality in patients with diabetes mellitus and COVID-19	Bronx, New York, United States	retrospective cohort	High
16	Karampoo et al.	The role of lovastatin in the attenuation of COVID-19	Iran	case–control	Fair

Table 1.
Observational studies.

Tan et al. conducted a retrospective cohort study within a Singaporean tertiary center and evaluated the association between antecedent statin use and a number of clinical outcomes within COVID-19 patients [47]. They used logical treatment models with PSM to compare statin users' (n = 40) and non-statin users' (n = 509) risk of admission to the intensive care unit (ICU), hypoxia, mechanical ventilation, and death. A lower chance of ICU admission was independently associated with antecedent statin therapy (P = 0.028), while other clinical outcomes such as hypoxia requiring supplemental oxygen (P = 0.449), invasive mechanical ventilation (P = 0.114), and death (P = 0.488) did not significantly differ between statin and non-statin users.

In a retrospective single-center study, Vahedian-Azimi et al. evaluated the correlation between use of ACEis, ARBs, statins, and aspirin on the clinical outcomes of mortality and ICU admission in patients hospitalized with COVID-19 [48]. Atorvastatin therapy, specifically, was found to be associated with reduced mortality after adjusting for age, lockdown status, and other medications (P = 0.001).

Rodriguez-Nava et al. evaluated whether a daily statin dose of 40 mg reduced inpatient mortality due to COVID-19 in the ICU of a hospital located in Evanston, IL [49]. Confounding factors were minimized by adjusting for age, hypertension, cardiovascular disease, mechanical ventilation, disease severity, number of comorbidities, and other adjuvant therapies. The retrospective cohort study found that

Interventional studies					
#	**Authors**	**Title**	**Location**	**Study Design**	**RoB 2.0 Score**
1	Ghati et al.	Statin and aspirin as adjuvant therapy in hospitalized patients with SARS-CoV-2 infection: a randomized clinical trial (RESIST trial)	India	open label, randomized control trial	Low Risk
2	INSPIRATION-S Investigators	Atorvastatin versus placebo in patients with covid-19 in intensive care: randomized controlled trial	Iran	double-blind, randomized control trial	Low Risk
3	Ghafoori et al.	Survival of the hospitalized patients with COVID-19 receiving atorvastatin: A randomized clinical trial	Bojnurd, Iran	single-blind, randomized control trial	Low Risk
4	Matli et al.	Managing endothelial dysfunction in COVID-19 with statins, beta blockers, nicorandil, and oral supplements: A pilot, double-blind, placebo-controlled, randomized clinical trial	Lebanon	double-blind, randomized control trial	Low risk

Table 2.
Completed interventional studies.

atorvastatin was associated with the slowest progression to death out of several target interventions [adjusted HR = 0.38; 95% confidence interval (CI) = 0.18–0.77; P = 0.008] when performing a multivariable Cox proportional hazards model. Additionally, non-statin users were shown to have a 73% faster progression to death.

In a retrospective multicenter cohort study conducted in Belgium, Spiegeleer et al. explored the association of antecedent statin and/or ACEi/ARB use with severity of symptoms and clinical outcomes in older adults infected with COVID-19 across two nursing homes (n = 154) [50]. Logistic regression models were utilized while adjusting for covariates of age, sex, functional status, diabetes, and hypertension. Statin use was found to be significantly related to the absence of symptoms during infection [odds ratio (OR) = 2.91; CI = 1.27–6.71]. A positive association between statins and serious clinical outcomes, such as death within 14 days of disease onset and prolonged hospital admission, was also observed, though it was not statistically significant (OR = 0.75; CI = 0.24–1.87).

Oh et al. conducted a population-based cohort study in South Korea to investigate whether prior and inpatient statin therapy affected COVID-19 incidence and hospital mortality in patients with COVID-19 [51]. Logistic regression analysis with PSM revealed that among 122,040 adults, statin users (n = 22,633) were 35% less likely to develop COVID-19 than non-statin users (n = 101,697) (OR = 0.65; 95% CI 0.60–0.71, p < 0.001). Hospital mortality in COVID-19 patients, however, did not differ between the statin and control groups when applying the multivariable model (OR = 0.74; 95% CI = 0.52–1.05; P = 0.094).

A nationwide observational study conducted in France by Cariou et al. sought to evaluate the association between routine statin therapy and clinical outcomes of COVID-19 inpatients across 68 hospitals with Type 2 diabetes mellitus (T2DM) [52].

#	Trial Identifier	Trial Phase	Study Design	Title	Sponsor/ Institution	Location
1	NCT04952350	III	Quadruple blinded placebo-controlled randomized trial	COVID-STAT	Mansoura University	Egypt
2	CTRI/2021/04/032648	III	Randomized, Parallel Group Trial	NAAC	Dr. Ambudhar Sharma	India
3	NCT04472611	III	Open-label, parallel assignment randomized trial	COLSTAT	Yale University	United States
4	NCT04466241	IIb	Open-label, parallel assignment randomized trial	INTENSE-COV	ANRS, Emerging Infectious Diseases	Côte D'Ivoire
5	NCT04380402	II	Open-label, parallel assignment randomized trial	STATCO19	Mount Auburn Hospital	United States
6	NCT04348695	II	Open-label, parallel assignment randomized trial	Ruxo-Sim-20	Fundación de investigación HM	Spain

Table 3.
Ongoing clinical trials.

Logistic regression analysis via inverse probability of treatment weighting (IPTW) with propensity score weighting was performed. The primary outcomes selected for were tracheal intubation and/or death within 7–28 days of admission. Patients who received antecedent statin therapy (n = 1192) were shown to have similar primary outcome rates as non-statin users (n = 1257) within 7 (29.8 versus 27.0%, respectively; P = 0.1338) and 28 days of admission before adjustment (36.2 versus 33.8%, respectively; P = 0.2191). After IPTW application, there was a significant association observed between statin therapy and intubation within 7 days (OR = 1.38; 95% CI = 1.04–1.83). Antecedent statin use was also significantly associated with a lower likelihood of death within both 7 (OR = 1.74; 95% CI = 1.13–2.65) and 28 days (OR = 1.46; 95% CI = 1.08–1.95).

In Wuhan, China, Fan et al. conducted a retrospective case study to investigate the association of statin use and in-hospital outcomes of 2147 patients admitted with COVID-19 across two hospitals [53]. Using a multivariate Cox model, statin users (n = 250) showed a lower risk for mortality (HR = 0.428; 95% CI = 0.169–0.907; P = 0.029), ARDS (HR = 0.371; 95% CI = 0.180–0.772; P = 0.008), and ICU admission (HR = 0.319; 95%; CI = 0.270–0.945; P = 0.032) than non-users (n = 1897) when

adjusted for age, gender, admitted hospital, comorbidities, inpatient medications, and blood lipids. Similarly, before and after adjusting for covariates, a Cox regression model revealed lower mortality (unadjusted HR = 0.254; 95% CI = 0.070–0.926; P = 0.038), ARDS development (unadjusted HR = 0.240; 95% CI = 0.087–0.657; P = 0.006), and ICU admission (unadjusted HR = 0.349; 95% CI = 0.150–0.813; P = 0.015) to be linked with statin therapy. A 1:1 matched cohort (206:206) was additionally created with PSM analysis of 18 potential confounders and showed statin use to be associated with better survival on a Kaplan–Meier survival curve (P = 0.025).

Peymani et al., in their retrospective cohort analysis, studied the role of routine and inpatient statin therapy in 150 patients hospitalized with COVID-19 in the Shiraz province of Iran [54]. The association of statin therapy and rate of death was assessed using Cox proportional hazards regression models. They found statins were associated with lower risk of death (HR = 0.76; 95% CI = 0.16–3.72; P = 0.735) and lower risk of morbidity (HR = 0.85; 95% CI = (0.02, 3.93), P = 0.762), although these results were not statistically significant. Statin therapy also reduced the chances of mechanical ventilation (OR = 0.96; 95% CI = 0.61–2.99; P = 0.942) and abnormal CT scan results (OR = 0.41; 95% CI = 0.07–2.33; P = 0.312). However, these findings were also not statistically significant.

Israel et al. performed a population-based cohort study with data collected from Clalit Health Services, the largest healthcare provider in Israel, to investigate the protective effects of statins, as well as several other established drugs, on COVID-19 hospitalization [55]. They utilized two case–control matched cohorts to assess which medications had the greatest protective effect. Five control patients chosen from the general Israeli population were matched to each case (n = 6202) in the first cohort and each case in the second cohort (n = 6919) was matched to two non-hospitalized SARS-CoV-2 positive control patients. In regards to our drug of interest, routine rosuvastatin use was identified as one the therapies that most significantly reduced risk of hospitalization due to COVID-19 (OR = 0.673; 95% CI = 0.596–0.758; P < 0.001) in both cohorts. Furthermore, pravastatin use was significantly associated with lower hospitalization risk (OR = 0.673; 95% CI = 0.493–0.902; P = 0.00659) in cohort 1. Similar effects were not observed with other statins.

A retrospective study conducted by Ayeh et al. analyzed the relationship between statin use and COVID-19 clinical outcomes, defined as prolonged hospital stay (≥ 7 days) and/or invasive mechanical ventilation, in patients admitted at the Johns Hopkins Medical Institutions across Maryland, United States [56]. Univariable and multivariable analyses were performed via logistic regression, Cox proportional hazards regression, and PSM. After Cox proportional regression application, statin use showed a protective effect against hazard of death (HR = 0.92; 95% CI = 0.53–1.59), though this was not statistically significant. Historical statin use was not found to reduce duration of hospitalization or need for intubation (relative risk (RR) = 1.00; 95% CI = 0.99–1.01; P = 0.928). Rather, it was associated with an 18% increased risk of severe infection (RR = 1.18, 95% CI = 1.11–1.27; P < 0.001).

In Milan, Italy, Bifulco et al. performed a retrospective cohort study on prior and in-hospital statin therapy as related to COVID-19-induced mortality in patients admitted to Humanitas Clinical and Research Hospital (n = 541) [57]. When adjusting for confounding variables such as age, gender, and pre-existing comorbidities, the odds of all-cause mortality were shown to be insignificantly lower in statin users (n = 117) compared to non-users (n = 424) (adjusted OR = 0.75; 95% CI = 0.26–2.17; P = 0.593). Though also not statistically significant, the risk of all-cause mortality was 14% lower in statin users when excluding the 12.4% of patients (n = 67) who

discontinued statin treatment due to tracheal intubation (adjusted OR = 0.86; 95% CI = 0.28–2.63; P = 0.795).

Marić et al., in their retrospective cohort study, evaluated 18,466 COVID-19 patients across 62 United States healthcare centers within the COVID-19 electronic health record (EHR) database of Cerner Real-World Data to analyze the effect of statin therapy on mortality rates of inpatients who were not previously prescribed statins [58]. PSM utilizing a 1:2 ratio and nearest neighbor method of patient demographics, comorbidities, and medication indication was used to compare the statin group (n = 2297) to the control (n = 4594). Statin drugs included were the following: atorvastatin (Lipitor), cerivastatin (Baycol), fluvastatin (Lescol), lovastatin (Mevacor), pitavastatin (Zypitamag, Livalo or Nikita), pravastatin (Pravachol), rosuvastatin (Ezallor or Crestor), simvastatin (FloLipid or Zocor). In the 10 cases of PSM by all three factors, a small but statistically significant reduced mortality rate was observed in patients who were prescribed statins (18.0%) compared to the matched controls (20.6%) (P < 1.00E-04 for all iterations).

A retrospective single-center study conducted by Saeed et al. enrolled COVID-19 patients with diabetes mellitus (DM) admitted to a hospital in Bronx, New York to compare risk of death during hospitalization between those who did and did not receive statin therapy [59]. Analysis via competing events regression revealed statin use (n = 983) to be associated with a 15% decrease in hospital deaths of patients with both DM and COVID-19 when compared with non-statin use (n = 1283) (24% versus 39%; P < 0.01). There was no difference in mortality observed from statin to control groups without DM (20% versus 21%; P = 0.82). In addition, PSM (HR = 0.88; 95% CI = 0.83–0.94; P < 0.01) and IPTW (HR = 0.88; 95% CI = 0.84–0.92; P < 0.01) were used to limit potential confounders, both of which showed a 12% reduced risk of in-hospital death for statin users.

In their single-center case–control study, Karampoor et al. analyzed the protective effects of lovastatin on 284 patients with severe COVID-19 admitted to the ICU of Firouzgar Hospital in Iran [60]. Participants comprised of three groups: (1) a control group of patients who did not receive lovastatin therapy (n = 92), (2) patients who received a daily dose of 20 mg lovastatin (n = 99), and (3) patients who received 40 mg lovastatin per day (n = 93). Blood samples were tested on the first day of hospitalization (T1), 3 days after hospitalization (T2), and 6 days after hospitalization (T3) in order to analyze dynamic changes upon inflammatory markers. Both lovastatin test groups exhibited statistically significantly reduced CRP, IL-6, and IL-8 levels between T1 and T3 (P < 0.05). Changes in IL-6 and IL-8 levels were found to be dose dependent; the 40 mg lovastatin group had significantly more reduced levels than the 20 mg group. Furthermore, the length of hospitalization of patients who received lovastatin was significantly lower than the control (P < 0.05). Mortality rate, however, did not statistically differ between test and control groups (P > 0.05).

3.2 Interventional studies

Ghati et al. published the results of the RESIST trial, a single-center, prospective, four-arm parallel design, open-label randomized clinical trial (RCT), that investigated the potential effects of novel statin and/or aspirin treatment on clinical deterioration and inflammatory response of patients requiring hospitalization for mild to moderate COVID-19 [61]. A total of 900 COVID-19 patients diagnosed via reverse transcription polymerase chain reaction (RT-PCR) were randomized into Group A (n = 224) to receive 40 mg of atorvastatin daily, Group B (n = 225) to receive 75 mg

of aspirin daily, Group C (n = 225) to receive conjunctive atorvastatin and aspirin therapy, or Group D (n = 226) to receive the standard of care (SOC). All participants were treated with SOC in addition to statin and/or aspirin therapy, save for Group D, which received solely SOC. Groups were followed for 10 days of hospitalization or until discharge, whichever came first. The primary outcome of clinical deterioration was characterized via WHO Ordinal Scale for Clinical Improvement (WHO-OSCI) ≥ 6 and secondary outcome inflammatory response was evaluated via changes in serum inflammatory markers of CRP, IL-6, and troponin I from time zero to day 5 of enrollment. Modified intention-to-treat (ITT) analysis revealed no significant difference in WHO-OSCI among the four groups (P = 0.46). Additionally, the primary outcome was not reduced when comparing all study participants who received atorvastatin (n = 442; HR = 1.0; 95% CI = 0.41–2.46; P = 0.99) and aspirin (n = 442; HR = 0.7; 95% CI = 0.27–1.81; P = 0.46) to the control (n = 219). Secondary outcomes of serum troponin I (P = 0.55) and CRP (P = 0.89) levels showed no significant changes between interventional and conventional groups, while IL-6 levels exhibited a significant decrease with conjunctive therapy (Group C; P < 0.001) and aspirin-only therapy (Group B; P = 0.04).

A double-blind, multicenter RCT with a 2×2 factorial design known as INSPIRATION/INSPIRATION-S was conducted by Bikdeli et al. across 11 hospitals in Iran to compare clinical outcomes of ICU-admitted COVID-19 patients who received statin treatment versus a placebo [62]. Participants, all of whom had no prior indication for statin use, were randomly assigned in a 1:1 ratio to receive atorvastatin 20 mg daily (n = 303) or a matching placebo (n = 302) in addition to SOC for 30 days from the time of randomization or until the primary outcome was reached. The primary outcome of interest was a composite of venous or arterial thrombosis, extracorporeal membrane oxygenation, and all-cause mortality. The individual components of the primary outcome as well as the number of days in which mechanical ventilation was not required comprised the secondary outcome. The median duration of use of atorvastatin therapy was 21 days and placebo was 19 days (P = 0.79). The primary outcome was observed in 33% (n = 95) of the interventional group and 36% (n = 108) of the placebo group (OR = 0.84; 95% CI = 0.58–1.21; P = 0.35), which indicated a risk difference of −3.6% (95% CI = −11.2–4.0%). Of the composing factors, all-cause mortality contributed the largest impact as the risk of mortality was 31% (n = 90) in the interventional group and 35% (n = 105) of the placebo (OR = 0.84; 95% CI = 0.58–1.22). No significant differences between atorvastatin and placebo groups were found in the incidence of venous (P = 0.64) or arterial thromboembolism (P = 0.32). There were no patients from either study group that required extracorporeal membrane oxygenation treatment. Similarly, both interventional and placebo groups had a 30-day median duration of ventilator-free days (P = 0.08).

An single-blind RCT was conducted by Ghafoori et al. in 2021 in Bojnurd, Iran, on patients hospitalized with COVID-19 [63]. In this RCT, 156 participants were randomized in a 1:1 ratio into a comparison group, which received standard therapy of hydroxychloroquine 400 mg daily and lopinavir/ritonavir 400/100 mg every 12 hours, or an interventional group, which received atorvastatin 20 mg daily plus SOC. All patients were followed until discharge or death, whichever result occurred first. Survival analysis, via Cox proportional-hazards regression and Kaplan–Meier analysis, was implemented to determine the primary outcome of hospitalization duration. Other outcomes studied included ICU admission and paraclinical findings. Atorvastastin exhibited a significant association with increased mean length of

hospitalization (7.72 days versus 5.06 days; P = 0.001) and heart rate (94.26 versus 87.87 per minute; P = 0.004) in comparison to SOC. Admission to the ICU was also higher in patients who received atorvastatin (18.4 versus 1.3%; P = 0.001) and the comparison group had a higher remission probability as evidenced by Kaplan–Meier analysis (P = 0.0001). After applying Cox regression analysis to adjust for age, length of hospital stay remained significantly reduced (HR = 1.70, 95% CI = 1.22–2.38; P = 0.002) and remission occurred 1.71 times sooner in the control group (HR = 1.70; 95% CI = 1.22–2.38; P = 0.002).

MEDIC-LAUMC was a small double-blind, placebo-controlled RCT conducted by Matli et al. that tested the efficacy of a five-drug protocol consisting of nicorandil, L-arginine, folate, nebivolol, and atorvastatin in the treating endothelial dysfunction in patients hospitalized with mild, moderate, and severe COVID-19 [64]. Thirty seven patients were assigned 1:1 to either the endothelial interventional group (n = 17) or placebo group (n = 20) and received their corresponding medications for 14 days. They were followed for an additional 14 days (28 days total) in hospital or as outpatients to observe outcomes. In the interventional group, participants were given 40 mg atorvastatin daily (unless previously on a statin, in which case they continued their current regimen), folic acid 5 mg daily, L-arginine 1 g thrice daily, nicorandil 10 mg twice daily, and nebivolol 2.5 or 5.0 mg once daily, depending on each patient's heart rate upon enrollment. The primary outcome was duration until recovery, measured by WHO-OSCI, and secondary outcomes were all-cause mortality, ICU admission, and need for invasive mechanical ventilation. There was no significant association found between the combination drug and median recovery time (P = 0.854), ICU admission, or need for mechanical ventilation (P = 0.644). Furthermore, no deaths were observed in either study group during the 28-day follow up period.

3.3 Ongoing trials

The phase III COVID-STAT is a single-center, double-blinded, RCT in Egypt that is expected to enroll 220 participants who are hospitalized for COVID-19 [65]. The trial will compare the efficacy of atorvastatin 40 mg orally for a maximum of 28 days with a placebo control arm. The main goal is to analyze all-cause mortality data of atorvastatin therapy after 28 days and 6 months from initial randomization.

Another phase III RCT, NAAC, is an open-label study in India that is measuring the efficacy of a new combination drug therapy consisting of aspirin, atorvastatin, and nicorandil in moderate–severe cases of COVID-19 [66]. Progression of In-hospital mortality, length of hospital stay, (invasive and non-invasive) mechanical ventilation, ARDS, thrombotic and cardiac events, and acute kidney injury are all parameters that define primary outcomes for this study.

More recently, COLSTAT is a phase III open-label, multicenter trial conducted by Yale University that aims to evaluate the effect of colchicine/rosuvastatin combination therapy in hospitalized COVID-19 patients [67]. 1:1 randomization will be used to assign 466 patients to an interventional group, to receive combination therapy in addition to SOC, or conventional group, to receive solely standard therapy. Patients in the interventional group will be administered rosuvastatin 40 mg daily and a loading dose of 0.6 mg colchicine twice daily for 3 days, after which they will continue on a maintenance dose of 0.6 mg colchicine daily for the duration of hospitalization or 30 days. Progression to severe COVID-19 as defined by WHO-OSCI 5–8 and arterial and/or venous thromboembolic complications are the primary outcomes to

be studied. Other outcomes include 30-day composite of death, respiratory failure requiring intubation, and myocardial injury.

In Côte d'Ivoire, an ongoing phase IIb open-label RCT (INTENSE-COV) is studying the benefits of using atorvastatin and telmisartan in combination with antiviral treatment, lopinavir/ritonavir to reduce viral load from the patient's body and thereby improve clinical outcomes [68]. The three arms include SOC (lopinavir/ritonavir 200/50 mg for 10 days), lopinavir/ritonavir 200/50 mg plus telmisartan 40 mg for 10 days, and lopinavir/ritonavir 200/50 mg plus atorvastatin 20 mg for 10 days. Outcome measures will be evaluated on serum levels of C-reactive protein and detection of SARS-CoV-2 virus on PCR from a nasopharyngeal swab. Clinical improvement based on WHO-OSCI, hospital duration, oxygen supplementation, endothelial activation markers, death rates, and adverse effects will also be indicated.

STATCO19, is a phase II single-center, open-label RCT in Cambridge, Massachusetts with 300 participants that aims to compare atorvastatin 40 mg orally for 30 days (plus SOC) against SOC in hospitalized patients with COVID-19 disease [69]. The primary outcome is the portion of patients who will progress to severe or critical status requiring ICU admission and/or emergency salvage therapy, or death, as measured by scores 5–8 according to the WHO-OSCI. Secondary measurements will include clinical outcomes on day 7 and 30 and also proportions of patients who test negative on day 7 based on PCR.

Ruxo-Sim-20 RCT is another phase II single-center study that is expected to include 94 participants in Madrid, Spain who are hospitalized for COVID-19 [70]. Patients will be divided into two groups: ruxolitinib 10 mg orally for 7 days and then 20 mg for 7 days plus simvastatin 40 mg orally for 14 days and standard of care. The trial aims to evaluate the percentage of patients who develop severe respiratory failure over a course of 7 days, as denoted by scores above 5 on the WHO-OSCI. Other outcomes may include length of ICU and hospitalization times, survival rates at 1, 6, and 12 months, and adverse events to the drug combination.

4. Discussion

To date, most literature that evaluate the initial efficacy of antecedent and inpatient statin therapy on the clinical outcomes of COVID-19 hospitalization have been epidemiological and observational in nature. We recognize that this may introduce several areas of biases, most commonly though short study duration or follow up period, absence of randomization, and small sample size. We attempted to mitigate these factors in this chapter by screening for a comprehensive list of studies with robust designs that were noted to be either of "High" or "Fair" quality on the Newcastle-Ottawa Scale for quality assessment.

Among the 16 observational studies, in-hospital mortality, invasive mechanical intubation, length of hospitalization, and ICU admission were the most common parameters analyzed. Statins were shown to significantly lower mortality rate in seven studies [45, 46, 48, 52, 53, 58, 59], reduce rates of ICU admissions in three studies [46, 47, 53], lower risk of invasive mechanical intubation in two studies [46, 52], and slow progression to death in two studies [49, 53]. However, several other studies revealed no significant benefit to statin use pre- or during hospitalization in improving clinical outcomes. Six studies demonstrated similar mortality data between statin and non-statin users [47, 50, 51, 54, 57, 60]. Three studies revealed no significant difference in incidence of mechanical ventilation [45, 47, 54]. One study found no effect of statin

therapy on the duration of hospital stay [50]. Surprisingly, one study even concluded that use of statins may contribute to adverse outcomes by increasing the rate of prolonged hospitalization and invasive ventilation in COVID-19 hospitalized patients [56].

Concerning the gold standard of RCTs, we described the findings from four publications that investigated the role of inpatient statin administration as a therapeutic for COVID-19. These trials were all determined to have "low risk of bias" when entered into the RoB 2.0 algorithm. The RESIST trial tested how statin therapy alone and in combination with aspirin affected clinical deterioration and inflammatory markers. Reportedly, only IL-6 levels significantly benefited from atorvastatin/aspirin conjunctive treatment [61]. The findings of the INSPIRATION/INSPIRATION-S trial showed no significant improvements of atorvastatin therapy in regard to venous or arterial thrombosis, extracorporeal membrane oxygenation, all-cause mortality, or requirement of invasive intubation [62]. Ghafoori et al. compared the standard of care for COVID-19 treatment to the experimental arm of atorvastatin with standard of care. Their results notably demonstrated that statin intervention actually significantly increased duration of hospitalization and pulse rate, as well as reduced likelihood of remission [63]. In another study, MEDIC-LAUMC found no significant improvement in median recovery time, rate of ICU admission, mechanical ventilation, or risk of mortality associated with the administration of a cocktail drug protocol that included nicorandil, L-arginine, folate, nebivolol, and atorvastatin [64]. Overall, there is currently little to no existing evidence from existing clinical trials that statin treatment has any benefit in preventing adverse clinical outcomes of COVID-19, and in one instance, it even contributed to worsened outcomes.

Finally, we reviewed ongoing clinical trials to assess future evidence impacting the use of statins in COVID-19. Of the six interventional studies described, three were in stage III [65–67], one was in stage IIb [68], and two were in stage II [69, 70]. Upon extensive search on the WHO International Clinical Trials Registry, ClinicalTrials.gov, and the NIH Clinical Center, no trials belonging to stage IV have been declared. Most prospective trials have been focused on testing the efficacy of statins as adjunctive therapy rather than a primary treatment option. Only two studies, COVID-STAT and STATCO19, are evaluating clinical outcomes associated with statin therapy alone (with standard of care) [65, 69]. Similar to published clinical trials, primary outcomes included parameters such as all-cause mortality data, ICU admission rates, intubation incidence, and progression of disease. Secondary outcomes were more concerned about serum levels, oxygen requirements, adverse effects, acute kidney injuries, and myocardial stress. While the gap in knowledge between COVID-19 treatments has certainly narrowed since the beginning of the pandemic, the therapeutic role of statins is not yet clear. There are still many questions regarding the efficacy of statins, especially on newer strains of SARS-CoV-2 like Omicron (B.1.1.529) or on post-COVID-19 syndrome. Other areas of investigation might also include a comprehensive assessment of lipophilic versus hydrophilic statins on treatment of COVID-19.

5. Conclusion

Statin drugs used alone or as adjuvant treatment for patients hospitalized with COVID-19 have revealed significantly marginal to inconclusive differences in clinical outcomes following review of multiple observational studies and clinical trials to date. Findings from forthcoming literature may provide robust evidence to further support this conclusion.

Conflict of interest

All authors have no disclosures or conflicts of interest.

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Chapter 8

Statins' Effects on Alzheimer's Disease

Qing Li, Chu-Na Li and Jing-Long Chen

Abstract

Alzheimer's disease (AD) has brought about heavy economic and healthy burden worldwide. There is no effective therapy to prevent or delay the progression of AD. Statins are suggested as the alternative therapy for AD, although the positive effects of statins on AD are still full of controversy. Therefore, it is necessary to define sensitive AD population who would benefit from statin therapy and a preferable therapeutic regimen on statins to avoid detrimental effects on cognition. We summarized the pathogenesis of AD, especially those related to statins. With emerging clinical evidence, updated data on the correlation between statins and AD development are clarified in chronological order. We also retrieved the underlying mechanisms for beneficial and detrimental effects of statins on AD development. Then we discussed the factors that might affect the efficacy of statins from statin use (types, dosages, and therapy duration) to the sensitive population (sex, age, genetic factors, and comorbidities). Finally, we elaborated on the limitations of the current studies and the implications for the future research to guide the appropriate statin therapy in clinic.

Keywords: Alzheimer's disease, statins, therapy, pathogenesis, mechanism

1. Introduction

Alzheimer's disease (AD) is a degenerative neurological disorder with high mortality and disability in the elderly. AD manifests cognitive decline in clinic and is characterized by cerebral deposition of amyloid-β (Aβ) plaques, tau neurofibrillary tangles, abnormal neuronal metabolism, neuronal cell death, and subsequent brain atrophy. As the most common form of dementia, nearly 5.8 million Americans were living with AD in 2020, and the population will exceed 150 million in 2050 worldwide, bringing about heavy economic and healthy burden [1].

It is crucial to explore the pathogenesis and potential targets for AD. One epidemiological study early in 1998 reveals the correlation between AD and high levels of serum cholesterol [2], followed by inconsistent result [3–5]. After pooling data on 23,338 patients, a recent meta-analysis suggests that high risk of AD in relationship with hypercholesterolemia happens in midlife and early stages of aging [6]. Thus, cholesterol-lowering agent might have a potential role in AD management.

Statins, 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitor, that effectively lower blood cholesterol, are widely used as the first-line agent for hypercholesterolemia in the primary and secondary prevention of cardiovascular events. Most

statins users are midlife or older adults with risk factors of cardiovascular and cerebrovascular diseases. Statins are reported to alter cognitive performance and AD development [7–9], although their positive role is still under debate [10, 11]. With more than 200 million people on statin therapy worldwide [1], it is critical to identify the role of statins on AD and explore the appropriate therapeutic plan for statins. Different from animal model studies, human studies produce inconsistent results. Some observational studies demonstrate that statins may prevent or delay the neurodegenerative process and reduce the risk of dementia or incident AD, especially in those who carry the APOE e4 allele or under 65 years old, while some other studies did not show any beneficial effects [12]. In fact, the mechanism involving statins in AD is complicated, including cerebral decomposition of Aβ and tau protein [13, 14], cerebral cholesterol balance [12, 15], neuroinflammation [14, 16], oxidative stress [13, 17], insufficient cerebral blood flow supply [18], abnormal blood-brain barrier [18], decreased neurotrophic factors [12, 19], and so on. Individual-related factors (including their genetic diversity, ethnicity, sex, age, and comorbidities) and statin-related factors (statin type, statin dose, and treatment duration) might alter the association between statin and cognition [12, 17]. Negative influence of statin on cognition might result from intracranial cholesterol depletion, reduced CoQ10, and decreased neurotrophic factor caused by statin therapy [12, 19].

Therefore, the identification of potential population who may benefit more from statin therapy and appropriate statin use might prevent or delay AD progress. We explored the pathogenesis of AD, especially those related to statins, and discussed the core position and related controversy of Aβ and tau protein. Then we reviewed the results of clinical research of statin therapy on AD, investigated underlying mechanisms for beneficial and detrimental effects of statins on cognitive performance, and analyzed potential factors modifying the cognitive effects of statins. Finally, we elaborated on the limitations of existing studies and the implications for future research to guide appropriate statin therapy.

2. Pathogenesis of AD

AD is a disease with complex pathogenesis, involving in gene and environmental factors. Amyloid-β (Aβ), tau protein, and neuroinflammation play leading roles in the development of AD [13].

2.1 The central position and challenges of Aβ and tau protein in AD

AD is characterized by cerebral accumulation of Aβ plaques, and tau neurofibrillary tangles. Aβ plaques are formed and deposited in different regions of the brain. These plaques are recognized as foreign material by the brain, which initiates an inflammatory and immune response by activating the microglia and releasing cytokines, eventually lead to neural cell death and neurodegeneration [20].

Aβ cascade theory was considered as the core of AD [21]. This theory holds that toxic Aβ fragments induce downstream damages: tau protein phosphorylation, neurofibrillary tangles, neuroinflammation, oxidative stress, neuron cell loss, and vascular damage, eventually leading to dementia. Besides Aβ, the over-phosphorylated tau protein also plays an important role, which affects the stability of the microtubule protein of the neuronal skeleton, leading to neurofibrillary tangle and destroying communication of neurons and synapses. More importantly, misfolding proteins of

Aβ and tau can "infect" and spread to surrounding tissues like the prion virus [21, 22]. In fact, the core position of Aβ cascade theory has been challenged. Positron emission tomography (PET) tracking in the living brain shows that tau protein is present in the brain of normal elderly. Aβ appears to trigger the expansion of tau depositions from the hippocampal formation to the limbic system, and subsequently to the neocortex. Significantly, it is tau protein rather than Aβ accumulation is consistent with the pathological process of AD, which implies that Aβ might only play an enzyme-linked role, and tau protein promotes the pathological changes of AD [23]. Therefore, when Aβ activates the downstream pathogenic pathway, it might be ineffective to target on Aβ. Unfortunately, clinical trials target on tau protein also failed [13].

2.2 Hypercholesterolemia

In 1998, Notkola *et al* reported a positive relation between hyperlipidemia and increased risk of AD in a cohort study [3]. Later, Solomon *et al* reached a similar conclusion in a larger study, showing a positive association between serum cholesterol levels in midlife and AD risk following 21 years [4]. However, Mielke *et al* did not find any significant association between midlife serum cholesterol levels and risk of AD following 32 years [5]. To explain the discrepancy of these results, a meta-analysis was performed and showed that the highest risk for developing AD due to hypercholesterolemia mainly happens in midlife and early stages of aging, not in late life [6].

Unlike epidemiology reports, experimental studies provide strong evidence for hypercholesterolemia and the risk of AD [17]. Diet-induced hypercholesterolemia significantly enhances cerebral neuroinflammation, Aβ plaque deposition and cognitive impairment in Wistar rats [17].

Statins, as a cholesterol-lowering agent, is found to reduce the content of cholesterol and oxidative cholesterol in the brain, as well as the levels of total cholesterol, latosterol, and 24S-OH chol in cerebrospinal fluid in animal and clinical studies [15]. In AD animal models, statins attenuate the formation of β-amyloid, reduce the production of senile neurotic plaques and neurofibrillary tangles, and improve cognition [24–27]. Simvastatin enhanced learning and memory performance in morris water maze test [28]. Lorenzoni *et al* made a similar observation in high-fat diet-feeding rats [29]. A meta-analysis of statin showed positive effects on cognitive function, especially in the younger mouse group [30]. Some observational studies make similar discoveries as animal studies, which demonstrated that statin treatment may prevent or delay the neurodegenerative process and reduce the risk of dementia or incident AD [8].

2.3 Cerebral vascular abnormality

Cerebral vascular alterations are discovered in over half of AD patients [18]. Patients with AD exhibit substantial atherosclerosis in leptomeningeal arteries and the circle of Willis [18]. In the late stage of AD, microvascular thinning and collapse are exhibited in up to 90% of the patients. Cerebral amyloid angiopathy (CAA) occurs in 85–95% of AD, where Aβ deposits in the outer membrane, middle membrane, and capillary basement membrane of intracranial arteries, leading to vascular wall damaging, causing micro infarction and micro hemorrhage, eventually cognitive decline [18].

Blood flow and metabolism decrease are discovered in cognitive-related brain areas (parietal, temporal, frontal lobes, especially and hippocampus) in AD patients through single-photon emission computed tomography (SPECT) perfusion imaging

and fluorodeoxyglucose PET imaging test. Even in the pre-clinical stage of AD, similar findings are discovered in cognitive-related brain areas (hippocampus, entorhinal cortex, amygdala, and anterior cingulate gyrus) [18]. Therefore, the roles of cerebral vascular abnormalities in AD pathogenesis might be underestimated.

2.4 Inflammation, oxidative stress, and other mechanisms

Neuropathology in human and animal studies confirmed that inflammation, oxidative stress, and immune regulation are involved in the pathogenesis of AD. Aβ deposits activate chronic inflammation and oxidative stress, which lead to the damage of neurons and dendrites and hinder intraneuronal communication [13, 20]. An abnormal gut microbiome causes systemic inflammation and neuroinflammation and is involved in AD progression via the brain-gut metabolic axis. Additionally, dysbiosis of the gut microbiome increased cytotoxic bile acid, which can cross the brain-blood barrier and deposit in the brain, leading to neuron apoptosis and neurodegeneration [17]. Synaptic dysfunction and neurotransmitter imbalance also participate in AD progression [20].

3. Clinical research of statins on AD

Epidemiologic studies suggest a close relationship between plasma hypercholesterolemia and AD risk [4, 6]. High serum cholesterol levels may alter sterol balance across the central nervous system [12]. Statins are wildly used in preventing and treating hypercholesterolemia, cardiovascular, and cerebrovascular diseases. Vasoprotective and neuroprotective effects are discovered in statin therapy in some studies [8, 10, 18, 31]. Therefore, the use of statins to reduce the risk of dementia and AD becomes a hot topic widely.

In 2000, Jick *et al* tried to explore whether modifying lipid burdens or components could lower the risk of developing dementia by using an observational approach. The results showed that individuals of 50 years and older who were prescribed statins had a substantially lowered risk of developing dementia but cannot distinguish between AD and other forms of dementia [10]. After these findings, interest has arisen in the potential for statins to delay cognitive decline or dementia in people with older age.

In 2006, a meta-analysis of statins for the prevention and treatment of dementia searched and sifted seven independent data sets from the literature over the last 40 years. They demonstrated that statins use did not show a beneficial effect on the risk of dementia or AD [11].

In 2013, Wong et al. conducted a meta-analysis of 15 observational studies of statins on dementia and AD. They observed a slight protective effect of statins in the prevention of AD and all-type dementia independently of the lipophilicity of the statins [8].

In 2013, Song et al. carried out another meta-analysis of eight prospective cohort studies including AD, vascular dementia and other dementia to examine the association of statins use with risk of dementia, which showed a significant association between statins use and a reduced risk of dementia, but no enough evidence for statins to delay the progression of AD [9].

In 2015, a review by McGuinness et al., including two double-blind, randomized, and placebo-controlled trials (HPS 2002 and PROSPER 2002), assessed the evidence of statins for the prevention of dementia. In these trials, simvastatin or pravastatin

were administered for at least 12 months to 26,340 people at risk of dementia. Both studies were at low risk of bias. Researchers discovered that initiating statin therapy in late life to people at risk of vascular disease do not prevent cognitive decline or dementia [32].

In 2018, Chu et al. conducted a systematic review and meta-analysis of 25 cohort studies published before 2017, and found that statins use significantly reduced the risk of developing all-cause dementia, AD, and MCI, but not incident VaD. Furthermore, statins may offer stronger preventative benefits for neurodegenerative dementia (such as AD) and MCI. Subgroup analyses suggested that both hydrophilic and lipophilic statins showed beneficial effects in preventing all-cause dementia and AD [33].

In 2020,Poly et al. performed a meta-analysis of 30 relevant observational studies from 2000 to 2018, including 9,162,509 participants (84,101 dementia patients). After fully adjusted in age, gender, and different types of covariates, statin use was discovered to be associated with a 17% decreased risk of all-cause dementia, and a 31% decreased risk of AD. North American individuals had a lower risk of dementia compared with Europeans and Asians [31].

Also in 2020, Xuan et al. conducted another meta-analysis including nine randomized controlled trials, to evaluate the efficacy of statins on AD. They discovered that statin therapy improved the scores of the MMSE scale in the short term ($\leq$12 months) but was not obvious after a longer time, and improved activities of daily living and the neuropsychiatric status, but not ADAS-Cog scale scores in AD patients. Statins appeared more effective in patients with high cholesterol levels and APOEε4 gene carriers [7].

Taken together, based on current evidence, statins seem to be correlated with a beneficial effect on AD patients, especially in those with high cholesterol levels and APOEε4 gene carriers, which requires future large RCT tests to validate.

4. Potential mechanism of statins on AD development

Statins may improve AD development by reducing intracranial Aβ and phosphorylated tau, improving cerebral blood flow and blood-brain barrier, decreasing inflammation and oxidative stress, and other mechanisms. The harmful effect of statins on cognition might be mainly attributed to excessive inhibition of intracranial cholesterol synthesis, which interferes with the formation of the neuronal cell membrane and myelin sheath, and impair synaptic function [34].

4.1 Statins reduce intracranial Aβ, phosphorylated tau

Statins regulate the activity of amyloid precursor protein (APP) related processing enzymes, decrease Aβ production and accumulation, and promote Aβ clearance, thus reducing intracranial Aβ levels [35–37], by MAPK/Erk1/2 [38], Rho/ROCK [39], or AKT/GSK3β signaling pathway [27].

Adult male guinea pigs fed with high doses of simvastatin for 3 weeks, showed a reduction of Aβ content in both brain tissue and cerebrospinal fluid, and the beneficial effects were reversed upon discontinuation of the drug [40]. AD mice model fed with atorvastatin and pitavastatin not only showed a reduction in intracranial Aβ and phosphorylated tau but also an improvement in cognitive function [26]. Cellular experiments by Yamamoto *et al*. showed that simvastatin and atorvastatin induced extracellular Aβ degradation by increasing neprilysin secretion from astrocytes through activation of MAPK/Erk1/2 pathways [38].

Statins reduce phosphorylated tau protein deposition. Boimel *et al*. demonstrated that either simvastatin or atorvastatin attenuated neurofibrillary tangles (NFTs) and improved memory in a transgenic mouse model of tauopathy. In addition, both lipophilic (simvastatin) and hydrophilic statin(atorvastatin) attenuated NFTs, suggesting that the ability of statins to cross the blood-brain barrier might not influence their effects on cognition [25]. Van der Kant et al. conducted a drug screening trial using neuronal cells generated by induced differentiation of pluripotent stem cells (iPSC) and found that statins (atorvastatin, simvastatin, fluvastatin, and rosuvastatin) reduced both Aβ secretion and phosphorylated tau protein deposition. However, they found that atorvastatin and simvastatin were toxic to astrocytes even at low concentrations, which needs to be confirmed by further studies [41].

4.2 Potential protection on cerebral blood flow, blood-brain barrier

Atorvastatin and pitavastatin can improve the blood-brain barrier by inhibiting the activation of MMP-9 and inflammatory reaction, thus attenuating neurovascular unit destruction in APP transgenic mouse models [42]. Tong et al. discovered that simvastatin improved cerebrovascular reactivity and counters soluble amyloid-beta, inflammation, and oxidative stress in aged APP mice [27]. One small sample-sized clinical study demonstrated that 4 months of atorvastatin increased CBF in bilateral hippocampi, fusiform gyrus, putamen, and insular cortices in persons at risk for AD [43], which should be validated by a large sample of clinical trials.

4.3 Anti-inflammatory and antioxidant effects

The anti-inflammatory and antioxidant effects of statins on AD have been confirmed in animal experiments [17, 27, 44, 45]. A great number of studies have shown that statins exert an anti-inflammatory and antioxidant effect through the activation of microglia and AGEs/NADPH oxidase/NF-kB signal pathway and thus, relieving neurodegeneration, and improving cognitive function [45, 46]. Statins not only reduce the pro-inflammatory mediators: the tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β), prostaglandin E2 (PGE2), interleukin-6 (IL-6), interferon-gamma (IFN-γ), cyclooxygenase-2 (COX-2), reactive oxygen species (ROS), and reactive nitrogen species (RNS) but induce the anti-inflammatory mediators such as interleukin 10 (IL-10) [12]. Simvastatin reduced Aβ-induced inflammation and oxidative stress [44]. Atorvastatin improved cognitive impairment by inhibiting inflammatory responses, suppressing Aβ-induced oxidative stress, and protecting mitochondrial function in hippocampal neurons [47, 48]. However, there are no clinical data on the effect of statins on cerebral inflammation and oxidation in AD patients.

4.4 Other mechanisms

Wnt/β-catenin signaling pathway is involved in the differentiation and maturation of dentate gyrus granulosa cells, dendritic branching, depolarization and formation, synaptic stability, and plasticity. Tong et al. showed that simvastatin activated Wnt/β-catenin pathway, subsequently rescuing memory and granule cell maturation in an AD mouse model [49].

Statins might affect memory performance by modulating the transcriptional activity of neurotrophic factors and related receptors. Simvastatin has been suggested

to enhance CREB (cAMP Response Element-Binding protein) and BDNF (Brain-Derived Neurotrophic Factor) in mice hippocampus, consequently improving memory functions [50, 51].

4.5 Harmful effect of statins on cognition

Some studies have shown the dementrial effect of statins on cognition. Hamano *et al* found that high doses of pitavastatin caused tau aggregation and even neuron apoptosis [39]. Treatment of elderly beagle dogs with human physiological doses of atorvastatin did not improve cognitive deficits and even resulted in transient reversible learning dysfunction [52]. Long-term combined use of simvastatin and four medications commonly prescribed to the Swedish elderly were found to impair explorative behavior and reduce synaptic functions in young adult mice [53].

The underlying mechanisms for statins-induced cognitive dysfunction may be mainly due to excessive inhibition of intracranial cholesterol synthesis, which leads to the depletion of intracranial cholesterol, thus interfering with the formation of the neuronal cell membrane and myelin sheath, and causing synaptic loss [12]. Furthermore, statins might induce oxidative stress and neuronal injury by reducing cerebral CoQ10 or BDNF levels [19]. The lower serotonin activity induced by statin might bring about behavior change that negatively influences cognitive performance [12].

5. Factors affecting the efficacy of statins

Statins therapy influences AD development through cholesterol-dependent or independent effects. Individual-related factors (age, sex, and genetic factors), statin-related factors (type, dosage, and duration), and comorbidities interfere with statins' effect on cognition.

5.1 Type, dosage, and treatment duration of statin

Whether lipophilic statins influence greater on AD progression more than hydrophilic statins is still inclusive. One meta-analysis reported that lipophilic statins were associated with a lower risk of AD [31]. On the contrary, one systematic review indicated that hydrophilic statins had more protective effects in preventing all-cause dementia and possibly AD in comparison to lipophilic statins [33].

Statins have a dose-dependent role in the prevention of cardiovascular and cerebrovascular events, but whether statins have a similar dose-dependent relationship in the prevention of dementia is still unclear. Tong et al. found that higher doses of simvastatin treatment improved both short-term and long-term memory in young adult AD mice, while a normal dose of simvastatin treatment (20 mg/kg/day for 8 weeks or 40 mg/kg/day for 3–6 months) failed to reverse intracranial Aβ deposition in AD mice [44, 54]. Wang S et al. found that high-dose (10 mg/kg/day) and long course of atorvastatin therapy prevented Aβ-induced neuroinflammatory responses, and improve impaired cognitive function; whereas the low-dose (5 mg/kg/day) atorvastatin did not show beneficial effects [55]. One meta-analysis showed that a 5-mg increase in the daily dose of statins resulted in an 11% decrease in dementia risk [56]. However, another two studies did not find any dose-dependent evidence between statins and dementia [57, 58].

Basic and clinical studies have found that only large or super doses of statins decrease intracranial cholesterol. Animal experiments showed that super doses of statins, such as lovastatin (100 mg/kg/day), pravastatin (100 mg/kg/day or 300 mg/day), and simvastatin (50 mg/kg/day), which is far above the clinical dosage, reduced the total amount of intracranial cholesterol and 24S-OH. In the clinical study, a high dose of statin (simvastatin 80 mg/day), rather than a regular dose of simvastatin (20 mg/day), decrease total cholesterol, lathosterol, and 24S-OH in cerebrospinal fluid [15]. Notably, intracranial cholesterol exhaustion induced by excessive lipid-lowering effect might lead to cognitive impairment [50]. So, it is possible to ensure a positive influence on cognition only when appropriately reducing cerebral cholesterol while maintaining cerebral cholesterol homeostasis.

As for treatment duration, a meta-analysis of rodent AD models found that a longer duration of statins(>6 months) got more benefit on Aβ deposition [24]. For patients at high risk of vascular dementia, more than 1 year of statin therapy significantly reduced the risk of dementia compared with nonusers [59]. However, one study with a follow-up of 10–37 years failed to show any associations between long-term statins exposure and cerebral amyloid or tau burden [60]. Since this research did not track cognition function, circulating lipid levels, and dose of statin, the result should be evaluated by further studies.

5.2 Age and sex

Aging is a major important risk factor for memory decline and AD. A study from the British Biological Sample Bank demonstrates that statin therapy favored individuals under 65 years old in cognitive function [61]. Volloch *et al*. recommended initiating statin therapy in the pre-clinical stage of AD [62]. The impact of sex in the effect of statins on AD and cognitive performance is still inconclusive, although a low level of estrogen in females serves as the main risk factor for AD [12]. Thus, future studies are required to decipher the statin cognitive effect concerning aging and sex.

5.3 Genetic factors

The apolipoprotein E ε4 allele (APOEε4) is the strongest genetic risk factor for AD. One longitudinal study of over 6 years has found that statin seems to attenuate memory decline in participants with heart disease and APOEε4 carriage [63]. Nevertheless, one autopsy-based research did not demonstrate any effect of APOE4 on AD pathological biomarkers in the statins exposure population [64], which needs to be carefully evaluated since the dosage and course of statin were not taken into account.

5.4 Comorbidities and renin-angiotensin system (RAS) system

Hypertension, ischemic heart disease, and stroke are high-risk factors for dementia. Those comorbidities might interfere with the outcome evaluation of statins research [12]. It is worth noting that neuroinflammation induced by chronic activation of the RAS system plays a role in the pathogenesis of AD. A recent meta-analysis including over 3 million individuals showed a reduced risk of dementia in subjects taking ARBs agents, which reduced the risk of any dementia by 22% as compared to other antihypertensive medication, especially in AD (27% reduction in risk) [65]. Barthold *et al* made further research and found the combination of ACEI or ARB agent and statin decreases AD risk [66].

6. Conclusion

With the aging of the population, age-related diseases have become the main killer threatening lives, including dementia, cardiovascular, and cerebrovascular disease. The main causes of mortality in AD patients are cardiovascular diseases and cerebrovascular diseases, similar to the general population [67]. So, the prevention of cardiovascular and cerebrovascular events should be put in the same position as a reversal of AD progress in AD patients. Statin, as the first-line agent to prevent cardiovascular and cerebrovascular events for the elderly, has been reported to potentially delay AD development, thus receiving extensive attention.

We summarized the current evidence of statin therapy on AD and provided the following suggestions. Firstly, great efforts are required to avoid the adverse effects of statins on cognition and identify suitable populations who may benefit from a statin. Secondly, patients with middle-aged or APOE4 carriers might benefit from statins, and women aged 65–75 years old should not be recommended to use simvastatin [68]. Thirdly, due to the limited evidence on brain cholesterol and cognition, it is unclear whether there is a safety threshold value for brain cholesterol in statin therapy. Finally, the combination of statins and other agents needs to be confirmed in future studies.

In conclusion, the positive evidence of statin's effects on AD have not been fully confirmed. Stratified studies revealed some potential factors, improving or deteriorating statin effect on cognition. Future studies are required to provide more evidence for the rational use of statins and define sensitive populations.

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Conflict of interest

The author declares that there are no conflicts of interest.

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Chapter 9

Early Signals of Motor Disorders and Pleiotropic Effects of Statins

Maria-Isabel Jimenez-Serrania

Abstract

The most spread drugs to treat dyslipidemia alone or with hypertriglyceridemia are statins. These active ingredients are considered safe and effective. But, with all drugs, there are adverse reactions related to them, in this case, muscular disorders such as myalgia and the complication of rhabdomyolysis. Furthermore, other adverse reactions are less studied but interesting to know, such as motor disorders. Pharmacovigilance tools must maintain the tracing of risks for effects that appear and search for positive signals; one of them is to analyze suspected adverse drug reactions of active ingredients reported through the international repository of the World Health Organization with an adaptation of data mining Bayesian methodology. Surprisingly, almost all positive motor signals are not stated as adverse drug reactions in technical factsheets and, at the same time, are related to some pleiotropic effects of statins. This chapter tries to summarize this evidence for specific pairs of statins and potential motor disorders for further investigation.

Keywords: statin, adverse reaction, motor disorders, pleiotropic effect, positive signals

1. Introduction

HMG-CoA reductase inhibitors, most known as statins, are active ingredients capable of blocking the endogenous synthesis of cholesterol with the intention to reduce the high levels of LDL-cholesterol in blood. They are prescribed for hypercholesterolemia, with or without hypertriglyceridemia [1].

The safety of these drugs has been studied and followed up over time. The low occurrence of severe adverse events (e.g., rhabdomyolysis and increased transaminases) and widespread use lead to a relaxation in the observation and prevention of other events, less frequent or severe but the quality of life of patients [2].

We must consider that one of the former statins, cerivastatin, was withdrawn due to an adverse drug reaction (ADR) classified as rare such as rhabdomyolysis with potential lead to kidney failure [3, 4]. So, it could be interesting to get inside potential rare or very rare ADRs with statins stated or not stated in fact sheets.

Most adverse reactions with statins reported in summaries of product characteristics (SPCs) as rare or very rare are associated with blood (anemia, thrombocytopenia), gastrointestinal disorders (constipation, abdominal pain, flatulence, dyspepsia, diarrhea, nausea, vomiting, pancreatitis), hepatobiliary disorders (cholestasis, hepatic failure), immune system disorders (anaphylaxis), musculoskeletal and

connective tissue disorders (myopathy, myositis, rhabdomyolysis, tendinopathy including rupture), nervous system (headache, paresthesia, dizziness, peripheral neuropathy), skin and subcutaneous disorders (angioneurotic oedema, dermatitis bullous), reproductive system and breast disorders (gynecomastia), sense disorders (visual disturbance, hearing loss), and general disorders (asthenia, fatigue) [3, 5].

But, beyond these last general disorders and musculoskeletal-related reactions, no one motor disorder appears as ADR in SPCs.

2. What motor disorders are candidates

VigiBase® is the unique World Health Organization (WHO) global database for suspected ADRs maintained by the Uppsala Monitoring Centre (UMC) since 1968. It is a starting point to offer a reference view of early signals of statin's adverse reactions related to motor disorders to consider in therapeutics and future clinical research.

The free-user interface VigiAccess™ of this database allows one to search for all data coming from over 110 countries, undersigning a statement of the responsibility for the appropriate use and interpretation of data [6]. Nowadays, free access to national and international reporting ADR databases is essential for investigating new signals and possible risks of drugs.

For the present review, there are included data of the entire chemical subgroup of the Anatomical Therapeutic Chemical (ATC) Classification System C10AA "HMG CoA reductase inhibitors"—atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin, and pitavastatin [7]. Signals for cerivastatin—withdrawn in 2001—are included and analyzed as of contrast.

In this database, it is plausible to approach the frequency of suspected ADR using the data mining methodology developed and is used by de UMC as the WHO Collaborating Centre for International Drug Monitoring that is Bayesian Confidence Propagation Neural Network (BCPNN) [8], extensions [9–13] and adaptations with a correct interpretation of the signals [14–18].

The R® free software v3.4.1. R [19] and PhViD® Package v1.0.8 [20] were applied to obtain positive signals. Details of the algorithm performed are reported in Appendix A.

Statin/ADR	Ataxia and related	Parkinson and related	Movement disorders and related
Cerivastatin			movement disorders
Atorvastatin		Parkinson's disease	
Fluvastatin	general		
Lovastatin	general		
Pitavastatin		Parkinsonism	
Pravastatin			eye movement disorder
Rosuvastatin		Parkinson's disease	
Simvastatin	general; cerebellar	Parkinsonism	

Table 1.
Positive signals for statins related to motor disorders reported as ADR in VigiAccess™ not stated in the summaries of product characteristics (SPCs).

Statin/ADR	akathisia	akinesia	cogwheel rigidity	joint stiffness	Musculo skeletal stiffness	muscle rigidity	fatigue	tremor
Cerivastatin		x						
Atorvastatin				x	x			
Fluvastatin								
Lovastatin								
Pitavastatin					x			x
Pravastatin						x		
Rosuvastatin					x			x
Simvastatin	x		x				x	x

Table 2.
Other positive signals for statins related to motor disorders reported as ADR in VigiAccess™ not stated in the summaries of product characteristics (SPCs).

All signals of statins related to motor disorders were extracted among the positive ones and categorized according to the Medical Dictionary for Regulatory Activities (MedDRA) [21]. The aggrupation for statin-positive signals obtained was related to similar pathology following MedDRA, e.g., ataxia (that includes general and cerebellar); Parkinson (that includes disease, Parkinsonism) (see Appendix B).

Almost the totality of the positive signals observed in this preliminary analysis is not reported in SPCs. Subgroups of positive signals were stratified and summarized considering signals of motor disorders in the following: ataxia, Parkinson, movement disorders, (see **Table 1**) and other symptoms related to motor disorder conditions such as akathisia, akinesia, cogwheel rigidity, joint stiffness, musculoskeletal stiffness, muscle rigidity, fatigue, tremor (see **Table 2**). Fatigue for pravastatin was the only one reported in fact sheets.

The following is an analysis of the evidence on the possible pleiotropic effects of statins related to the selected motor adverse reactions. All searches for evidence were made in the Medline database via Pubmed® [22].

3. Pleiotropic effects and positive motor disorders

There is wide evidence that statins reduce vascular events such as coronary atherosclerotic heart disease and ischemic stroke. This treatment for dyslipidemias is almost constitutive in people over 65 years of age, and more in people over 80 [23]. But increasing age gets an implicit risk factor for adverse events such as myopathy, cognitive impairment, or motor disorders related to pleiotropic effects.

3.1 Deficient synthesis of coenzyme Q and ataxia

In the preliminary analysis, simvastatin is the only one that showed a positive signal of cerebellar ataxia and elevated numbers for general ataxia. Followed in the distance by lovastatin and fluvastatin that present only signals of general ataxia, these two last ones can be used as a control in studies to differentiate general ataxia from cerebellar ataxia for statins. The rest of the statins do not appear to generate situations related to ataxia, a fact to consider in the prescription of patients with a risk of this condition.

Some studies focus on the influence of coenzyme Q10 (CoQ10) deficiency-related to motor symptoms such as Friedreich's ataxia, Parkinson's, and Huntington's diseases [24]. CoQ10 is an antioxidant component of oxidative phosphorylation in mitochondria. Due to farnesyl pyrophosphate being a critical intermediate for CoQ10 synthesis, blockage of this step by statins may be important in the occurrence of myopathy and also has been associated with encephalomyopathy, severe infantile multisystemic disease, cerebellar ataxia, nephrotic syndrome, and isolated myopathy [25].

There are case series of patients with cerebellar ataxia due to statins use, which the authors considered probably related to coenzyme Q10 deficiency [26]. But the exposures to CoQ10, statins, and vitamin E did not appear to influence the clinical progression of spinocerebellar ataxia within 2 years [27].

Additionally, decreased levels of Coenzyme Q-10 have been demonstrated in diseased myocardium and Parkinson's disease [25]. Again, statins interfere with the synthesis of coenzyme Q10, and its deficiency is related to motor symptoms [24].

3.2 Inhibition of statin metabolism and masking neuromuscular disorder

All statins, except pravastatin, are metabolized by the CYP450, any drug that induces or inhibits CYPs can alter statin levels. Pravastatin is eliminated virtually unchanged by phase II reactions (conjugation to increase water solubility). This situation leads to underdose or overdose of statins [28].

Ataxia is an ADR also observed with another drug as carbamazepine or ergotamine, following the addition of a CYP3A4 inhibitor.

It is well recognized that statins affect muscular tissue adversely but, at the same time, these agents may act as unmasking agents in asymptomatic patients with a latent neuromuscular disorder. Muscular symptoms or increased serum CK levels persisting after statin treatment discontinuation should alert the clinician to pursue further diagnostic evaluations for the detection of potential underlying neuromuscular diseases [29].

Also, this situation can occur in patients with central nervous system metabolic disorders; as reported in cases of acute ataxia coincident with statin onset in individuals with bipolar disorder [30].

3.3 Hydrophilic: lipophilic balance and Parkinson risk

In the preliminary analysis, the statins more related to Parkinsonism were simvastatin and pitavastatin and Parkinson disease (PD) for atorvastatin and rosuvastatin.

Due to that plasma (S)24-OH-cholesterol seems inversely linked to Parkinson's disease [31], it could lead to those higher levels of total and low-density lipoprotein cholesterol over time indicating a decreased PD risk [32].

The authors of a recent publication also consider that statin use may have a detrimental effect on baseline nigrostriatal dopamine degeneration and long-term outcomes in patients with Parkinson's disease [33].

The use of lipophilic statin (simvastatin, lovastatin, atorvastatin, fluvastatin, and pitavastatin) was associated with a higher risk of PD, and the stronger association in initial use suggests a facilitating effect [34], but the effect in long-term studies fades as the evidence of their benefits in Parkinsonism [35].

But hydrophobicity is a key determinant for blood-brain barrier penetrance, and this recently suggests that hydrophilic, but not lipophilic, statins may be associated with faster PD progression [36].

There is an ongoing randomized control trial "PD STAT" for simvastatin as a neuroprotective treatment for PD with no results posted at the present [37].

The reality is that in patients with Parkinson, statins are less used without clear evidence. In a study, it was observed that over 60% of recent-onset PD patients have high or medium cardiovascular risk, which is associated with a worse motor and cognitive phenotype, but statins are underused in these patients [38].

3.4 Dopamine levels and negative motor symptoms in schizophrenia

Some symptoms of Parkinsonism, such as slowness of movements, muscle rigidity, increased appetite, and decreased energy were the most common adverse effects described in a randomized control trial with lovastatin versus placebo in schizophrenic drug-treated patients [39].

Nonetheless, there is conflicting evidence around statins as adjuvant therapy in schizophrenic drug-treated patients. The named trial did not observe differences between lovastatin and placebo [39], but a posterior meta-analysis concluded statins could improve psychiatric symptoms, either positive symptoms or negative symptoms [40]. In a recent review, authors indicate that, in patients with schizophrenia, negative motor symptoms may be reduced by adjuvant statin therapy [41].

In this sense, it would be interesting to deeply study the effect of statins—simvastatin, atorvastatin, pitavastatin—in schizophrenia.

3.5 Exclusive eye of movement disorders and underdiagnosis of Huntington/ Parkinson disease

In the preliminary analysis, it was observed a unique positive movement disorder signal for pravastatin and eye movement disorder with high sensitivity. Perhaps this ADR could be used as an alarm for surrounding movement disease not diagnosed.

Concerning general movement disorders, it is difficult to extract conclusions from former studies [42] but now statins seem to be protective [43, 44].

Also, there is evidence of movement disorders that appear as ADR due to a delay in the diagnosis in patients with Huntington's disease with premotor symptoms [45] or Parkinson's disease [43].

3.6 Changes in electric transmission and motor neuropathy

A study of long-term statin use revealed an increased risk of peripheral neuropathy [46], ADR reported in fact sheets. Electrodiagnostic changes have been detected in motor and sensory nerves in nerve conduction studies of these patients. If motor nerves are affected the movement is compromised.

The authors consider that early detection of peripheral neuropathy and changing hypercholesterolemia treatment may prevent permanent nerve damage. They offer also as a reference that the assessment of neurological symptoms, like tingling, numbness, pain, tremor in the hands and feet, and unsteadiness during walking may be useful in the follow-up of the patients on long-term statin treatment.

3.7 Anti-inflammatory and immunosuppressor effect and less join pain/stiffness

Regarding to other positive signals related to motor disorders observed (akathisia, akinesia, cogwheel rigidity, joint stiffness, musculoskeletal stiffness, muscle rigidity,

Statin	ADR
Atorvastatin	Parkinson (disease)
	Others: joint stiffness; musculoskeletal stiffness.
Fluvastatin	Ataxia (general)
Lovastatin	Ataxia (general)
Pitavastatin	Parkinson (Parkinsonism)
	Others: Musculoskeletal stiffness*; tremor*.
Pravastatin	Movement disorders (eye movement disorder*)
	Others: Muscle rigidity*
Rosuvastatin	Parkinson (disease)
	Others: Musculoskeletal stiffness; tremor.
Simvastatin	Ataxia (general; cerebellar)
	Parkinson (Parkinsonism*)
	Others: akathisia*; cogwheel rigidity; fatigue; tremor.

High specificity and sensitivity.

Table 3.
List of early positive signals of motor and related disorders detected for each statin agent and proposed to priority clinical investigation.

fatigue, tremor), the signals with more sensitivity in the analysis were akathisia with simvastatin, muscle rigidity with pravastatin, and signals of musculoskeletal stiffness and tremor for the more recent included pitavastatin. Only fatigue is reported in SPCs of pravastatin. Fluvastatin and lovastatin are free of all these signals and can be candidates for statin interchange.

Some of these symptoms are shared with other co-morbidities as rheumatoid arthritis. There is evidence of the pleiotropic effects of statins on ameliorating rheumatoid arthritis activity and mediating clinically apparent anti-inflammatory effects in the related autoimmune inflammation, which lead the authors to recommend statins as a potent treatment for these patients [47].

Other investigators have established that associations between statin use, and poor physical functioning, and self-rated health may be explained by factors other than joint pain/stiffness, e.g., muscle pain [48]. So, the affectation of statins in these other motor symptoms would be complicated to differentiate from the known effect of myalgia by statin use rather than the real appearance of these conditions.

This deduction could be plausible with respect to akathisia, akinesia, cogwheel rigidity, joint stiffness, muscle rigidity, and musculoskeletal stiffness, but less simple to understand the relationship between fatigue and tremor. At the same time, there is no evidence that statin use was protective in essential tremor [49].

As summary, due to the scarcity of strong evidence, it is relevant to propose a list of each statin and motor disorders with potential pleiotropic correlation (**Table 3**).

3.8 Limitations of the study

In the preliminary analyses, values of specificity and sensitivity of the BCPNN methodology, it is known that are typically low (21). Nonetheless, it is acceptable with very high specificity and low but conservative sensitivity, as it can be observed with

typical positive signals of rhabdomyolysis and myopathy with statins, among others (see Appendix C).

Data analyzed are previous and not influenced by interactions with SARS-CoV-2 infections, pharmacological treatments, or vaccines.

4. Conclusions

In the sight of the evidence collected, statins—beyond are considered equivalent and interchangeable regarding efficacy—are different in the interaction with patients with specific comorbidities or risk factors. So, the prescription of an indiscriminate statin converts the possibility to suffer a motor disorder into an ADR random discovery.

According with the pleiotropic effects discussed, several motor signals detected and proposed to further investigation such as musculoskeletal stiffness and tremor for pitavastatin, musculoskeletal rigidity and eye movement disorder for pravastatin, and Parkinsonism and akathisia for simvastatin.

This review may perform as a reference to statin interchange in case of detecting any early motor ADRs, as well as a starting point for future research. In both cases, due to the low positive motor signals detected, fluvastatin and lovastatin were positioned as the safer candidates.

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Conflict of interest

The author declares no conflict of interest.

A. Preliminary analysis

A.1 Fundamentals of the method

This method to detect adverse drug reaction (ADR) signals was improved by Uppsala Monitoring Centre of the World Health Organization with an extension to the multiple comparison setting. The key estimator is the calculated Bayesian false discovery rate (FDR) and the threshold to a positive signal fixed in FDR < 0.05 [12, 13].

Obviously, adaptations of this methodology can be valuable and trustworthy with a correct interpretation of the signals [14]. The adaptation applied in this study consists in contrasts all the ADR of a specific ATC subgroup isolated from the integral database. In this case, the chemical subgroup C10AA "HMG CoA reductase inhibitors" [15] was considered in the analysis. This adaptation was previously applied and approved robustness and consistency with other specific groups of drugs [16–18].

All signals of statins were obtained. Those ADRs related to motor disorders were extracted among the positive ones and categorized according to the standard terminology used in VigiAccess™, in essence, high-level terms (HLT) including preferred terms (PT) of the Medical Dictionary for Regulatory Activities (MedDRA) [21].

A.2 Details of algorithm performed

In this analysis, the algorithm was performed with the following arguments: value of the relative risk (RR) proven to be higher than 1 (RR < 1); minimum number of cases per pair [drug-adverse reaction] to be potentially considered as a signal (N = 1); rule of decision for the generation of signals: false discovery rate (FDR); limit or threshold for the decision rule: FDR > 0.05; statistics used for ordering the drug-ADR pairs: posterior probability of the null hypothesis (post.H0); calculation of the distribution of the statistic of interest: by approximation to the normal distribution [8, 50] and using empirical estimation through Monte Carlo simulations (NB.MC = 10,000) [51]. The estimator of FDR < 0.05 and specificity (Sp) ≥0.99 are considered to interpret the results. Sensitivity (Se) values are typically low in the BCPNN approach [52], Se ≥0.20 is considered as reference.

The estimator FDR assures that at least 95% of the signals detected are positive (only 5% of false positives). Moreover, if the estimator of false negatives (FNR) is 50% or lower, it implicates that, at least, half of the signals rejected are effectively negative. In the results presented, all the FNR were lower than 49%.

B. Preliminary analysis. Detailed results of positive signals (FDR < 0.05; Specificity ≥0.99) of motor disorders related with statins reported as adverse drug reaction (ADR) in VigiAccess™ database and analyzed by a contrasted approach of Bayesian Confidence Propagation Neural Network (BCPNN) extended to the multiple comparison setting for active ingredients groups.

Interpretation of items: drug code: active ingredient reported; event effect: ADR reported; count: number of couples "active ingredient-ADR" reported; post.H0: posterior probability of null hypothesis; FDR: False Discovery Rate; FNR: False Negative Rate; Se: Sensitivity (* ≥ 0.20); Sp: Specificity.

Ataxia, cerebellar ataxia.

Drug Code	Event Effect	Count	Post.H0	FDR	FNR	Se	Sp
Fluvastatin	Ataxia	11	0.044	0.009	0.448	0.149	0.999
Lovastatin	Ataxia	39	0.000	0.000	0.482	0.023	1
Simvastatin	Ataxia	87	0.000	0.000	0.475	0.051	1
Simvastatin	Cerebellar ataxia	7	0.101	0.025	0.435	*0.198	0.995

Parkinsonism, Parkinson's disease.

Drug Code	Event Effect	Count	Post.H0	FDR	FNR	Se	Sp
Pitavastatin	Parkinsonism	3	0.119	0.032	0.431	*0.212	0.993
Simvastatin	Parkinsonism	20	0.104	0.026	0.434	*0.200	0.995
Atorvastatin	Parkinson's disease	63	0.045	0.009	0.448	0.149	0.999
Rosuvastatin	Parkinson's disease	54	0.000	0.000	0.474	0.055	1

Movement disorders; eye movement disorder.

Drug Code	Event Effect	Count	Post.H0	FDR	FNR	Se	Sp
Pravastatin	Eye movement disorder	v5	0.107	0.027	0.434	*0.202	0.995
Cerivastatin	Movement disorder	90	0.000	0.000	0,480	0.034	1

Other motor symptoms related: akathisia, akinesia, cogwheel rigidity, joint stiffness, musculoskeletal stiffness, muscle rigidity, fatigue, tremor.

Drug Code	Event Effect	Count	Post.H0	FDR	FNR	Se	Sp
Simvastatin	Akathisia	5	0.149	0.043	0.424	*0.237	0.990
Cerivastatin	Akinesia	4	0.136	0.038	0.427	*0.227	0.991
Simvastatin	Cogwheel rigidity	5	0.053	0.011	0.446	0.158	0.998
Pravastatin	Fatigue	626	0.004	0.000	0.466	0.087	1
Simvastatin	Fatigue	2174	0.002	0.000	0.468	0.078	1
Atorvastatin	Joint stiffness	211	0.071	0.016	0.442	0.174	1
Pravastatin	Muscle rigidity	12	0.146	0.042	0.424	*0.235	0.990
Atorvastatin	Musculoskeletal stiffness	610	0.016	0.003	0.458	0.115	1
Pitavastatin	Musculoskeletal stiffness	25	0.154	0.046	0.422	*0.243	0.989
Rosuvastatin	Musculoskeletal stiffness	416	0.000	0.000	0.477	0.043	1
Pitavastatin	Tremor	28	0.106	0.027	0.434	*0.201	0.995
Rosuvastatin	Tremor	400	0.009	0.001	0.461	0.103	1
Simvastatin	Tremor	394	0.024	0.004	0.455	0.126	0.999

C. Preliminary analysis. Detailed results of positive signals (FDR < 0.05; Specificity ≥0.99) of disorders referred in main manuscript related to statins reported as ADR in VigiAccess™ database and analyzed by a contrasted approach of Bayesian Confidence Propagation Neural Network (BCPNN) extended to the multiple comparison setting for active ingredients groups

Interpretation of items: drug code: active ingredient reported; event effect: ADR reported; count: number of couples "active ingredient-ADR" reported; expected count: couples "active ingredient-ADR" expected; post.H0: posterior probability of null hypothesis; n11/E: ratio between the count observed and the count expected of the corresponding couple; drug margin: number of reports of a drug; event margin: number of reports of an event; FDR: False Discovery Rate; FNR: False Negative Rate; Se: Sensitivity(* ≥ 0.20); Sp: Specificity.

Rhabdomyolysis.

Drug Code	Event Effect	Count	Post.H0	FDR	FNR	Se	Sp
Cerivastatin	Rhabdomyolysis	5219	0	0.000	0.488	0.001	1
Simvastatin	Rhabdomyolysis	4873	0.000	0.000	0.487	0.004	1

Transaminases increased.

Drug Code	Event Effect	Count	Post.H0	FDR	FNR	Se	Sp
Fluvastatin	Transaminases increased	128	0.000	0.000	0.487	0.010	1
Atorvastatin	Transaminases increased	787	0.000	0.000	0.477	0.048	1
Simvastatin	Transaminases increased	467	0.000	0.000	0.474	0.059	1

Myalgia.

Drug Code	Event Effect	Count	Post.H0	FDR	FNR	Se	Sp
Simvastatin	Myalgia	11,860	0.000	0.000	0.487	0.005	1
Fluvastatin	Myalgia	1588	0.000	0.000	0.487	0.007	1
Pravastatin	Myalgia	3209	0.000	0.000	0.485	0.014	1
Lovastatin	Myalgia	2278	0.071	0.016	0.442	0.174	0.997

Myopathy.

Drug Code	Event Effect	Count	Post.H0	FDR	FNR	Se	Sp
Lovastatin	Myopathy	499	0.000	0.000	0.487	0.006	1
Cerivastatin	Myopathy	566	0.000	0.000	0.486	0.010	1
Simvastatin	Myopathy	1327	0.000	0.000	0.485	0.014	1
Fluvastatin	Myopathy	171	0.000	0.000	0.479	0.035	1

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Section 3

Statin-Associated Side Effects

Chapter 10

Reviews on Statin-Associated Side Effects

Qiutong Wu, Lu Fang, Yujie Zhu and Lemin Zheng

Abstract

Statins are a class of drugs widely used worldwide to manage hypercholesterolemia and prevent secondary heart attacks. They have an important role in reducing morbidity and mortality in patients with cardiovascular disease. Due to their wide range of biological effects, some potential therapeutic effects of statins have also attracted increasing attention, such as the treatment of multiple sclerosis, systemic lupus erythematosus, Alzheimer's disease, and chronic liver disease. However, a major problem with these kinds of applications is that long-term use of statins also has certain adverse reactions. These adverse effects include liver injury, myopathy, new-onset type 2 diabetes, renal dysfunction, interstitial lung disease, and other reactions. This article mainly reviews the adverse reactions of statins in clinics, aiming to provide a reference for the clinical application of these drugs.

Keywords: statins, side effects, serum cholesterol, adverse reactions, LDL

1. Introduction

Statins are a class of widely used oral lipid-lowering drugs in clinical practice. At present, in terms of their pharmacokinetic and pharmacodynamic profiles, available statins show various characteristics. Despite the primary target of statins serving as the inhibition of HMG-CoA reductase (HMGR), the rate-limiting enzyme in cholesterol biosynthesis, many pleiotropic effects can be easily found in statins in the downstream of the mevalonate pathway. Although these pleiotropic effects of statins may be a cause for enthusiasm, there are many adverse effects that, for the most part, are unappreciated and need to be highlighted. These adverse effects may be relatively uncommon, considering the number of people worldwide who use statins daily, and the actual number of people affected becomes quite large. In this overview, we mainly focus on the potential adverse effects of statins (**Figure 1**).

2. Adverse events of statins

Even though statins are prominently used in treating hypercholesterolemia and boast lots of pleiotropic effects, they also bear plenty of dose-dependent adverse effects. Therefore, it needs to be treated with caution when extensively utilized.

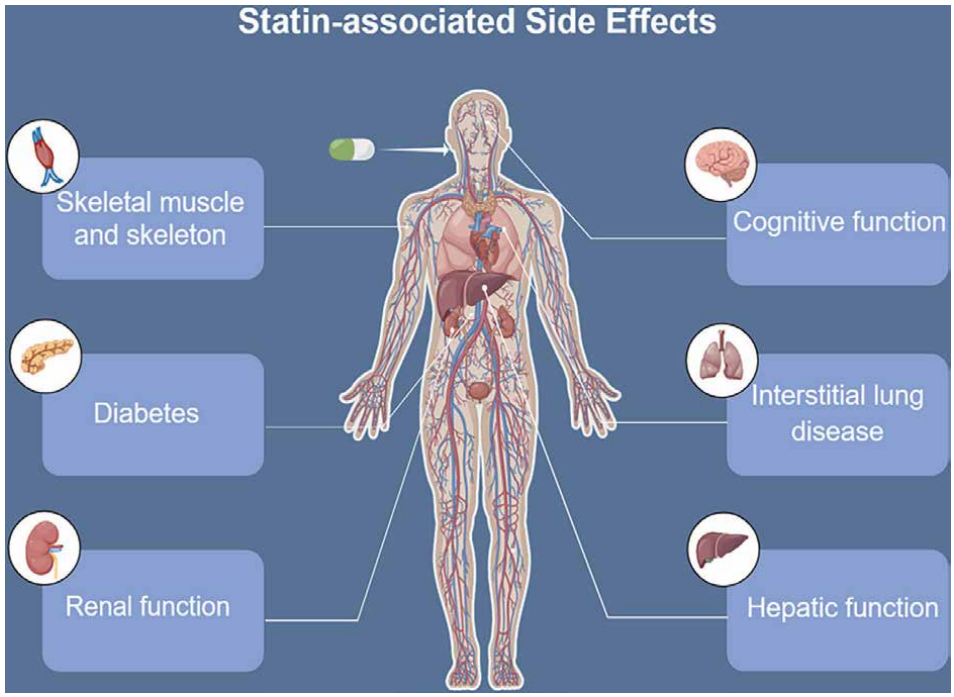

Figure 1.
Statin-associated side effects mainly include liver injury, skeletal muscle impairment, new-onset diabetes, cognitive function, renal dysfunction, and interstitial lung disease.

Statin-associated liver injury, muscle symptoms, new-onset type 2 diabetes, cognitive and renal dysfunctions, interstitial lung disease, and other reactions are among them. We aim to underscore these potential effects, some of which can be life-threatening.

2.1 Liver injury

In early clinical trials, the elevation of aminotransferase levels caused by the use of statins raised concerns about statin hepatotoxicity, which severely limited the clinical application of statins. There is no distinction between statin type and the incidence of transaminitis. Transaminitis may be related to changes in the hepatocyte membrane. When the lipid concentration of the hepatocyte membrane decreases, the cellular membrane subsequently becomes more permeable and allows lipophilic enzymes to leak [1].

The use of statins can also be associated with rare but severe liver injury and even sometimes severe hepatotoxicity. After recovery, similar symptoms of liver injury may recur in re-episodes. Liver injury develops in most patients 3–4 months after treatment. The statins, such as atorvastatin and simvastatin, are most commonly tied in with drug-induced liver injury (DILI). And only the liver damage caused by atorvastatin and simvastatin among statins has been linked to a catastrophic outcome. That is probably because these are the most utilized statins. Atorvastatin-associated liver injury has decreased biliary flow from the liver to the duodenum, whereas simvastatin has been associated with hepatocellular injury [2]. The likelihood of statin-induced hepatotoxicity may probably rise if the other hepatotoxic substances, such as alcohol, calcium channel blockers, and fibrates, are used in combination

with statin therapy [3]. Although liver injury can occur in patients treated with statins, actually this is rare [4]. In addition, there is evidence that specific liver injury caused by statins is dose-independent and is not more common in patients with liver disease than in other patients [5]. Whether statins can cause specific drug-induced liver injury in patients with chronic liver disease is not clear and still needs further research [5]. Patients, even though with potential liver pathology, are also supposed to be treated with statins as long as they have a solid indication. That is because many of these patients have a higher risk of cardiovascular death than those with liver disease [4].

2.2 Skeletal muscle impairment

Approximately 10–15% of patients experience different degrees of adverse reactions, such as myalgia, after taking statins. Muscle pain often causes patients to stop taking statins, which is known as statin intolerance. Myopathy, including myalgia, is a common dose-dependent side effect associated with statins. It is characterized by stiffness, cramps, weakness, or loss of strength during exertion [6]. The muscular toxicity of statins is manifested as muscle pain and aching (myalgia), myositis, and rhabdomyolysis, depending on the presenting symptoms and levels of creatine kinase (CK) [7].

Myopathy is defined as symptomatic muscle pain in which CK is elevated to greater than four times the upper limit of normal value (ULN), while severe myopathy is classified as muscle symptoms with CK between 10 and 50 times ULN [8]. There are no validated tests or clinical criteria, except for increases in CK, but CK increases are absent in most myalgia patients. Statin-associated muscle symptoms (SAMSs) can occur without creatine kinase (CK) elevations, and this is the most frequent SAMS presentation. Therefore, the diagnosis of SAMS is difficult and only based on clinical criteria.

Additionally, myalgia may be associated with statin treatment, which could be linked to reduced coenzyme Q10 (CoQ10) in skeletal muscle and impaired mitochondrial function. In the mitochondria, CoQ10 is an essential electron carrier in the electron transfer system. Therefore, a lack of muscle CoQ10 may impair the function of mitochondrial respiratory chain and increase the production of reactive oxygen species (ROS) [9]. Previous studies have found that mitochondrial respiratory dysfunction and increased ROS production are related with nociceptor activation and pain [10]. Therefore, although not supported by the literature, the link between statins, low levels of muscle CoQ10, mitochondrial dysfunction, and myalgia is biologically probable. Study found no differences in muscle CoQ10 levels or mitochondrial function in statin users with or without myalgia. Individual variations in muscle CoQ10 levels were not associated with variations in myalgia intensity in statin users with mild-to-moderate myalgia. Further studies are warranted to create methods to alleviate myalgia for statin users [11].

Recently, a study identified that in statin-treated human and rat, statin leads to the fact that the FK506-binding protein (FKBP12) dissociates from the ryanodine receptor 1 (RYR1) in skeletal muscle. This relates to increased unwarranted calcium release sparks. Nevertheless, despite the calcium sparks relating to upregulation of pro-apoptotic signaling markers (caspase-3 and the proportion of TUNEL positive nuclei), statins had no influence on muscle force production. So other factors are likely required for myotoxicity. And moderate exercise may mitigate the effects of statins on skeletal muscle [12].

Clinically important muscle symptoms, including rhabdomyolysis and statin-induced necrotizing autoimmune myopathy (SINAM), are rare. Particularly, rhabdomyolysis is the most feared complication of statin use. The risk of statin-induced rhabdomyolysis increases with age, administration of interacting drugs (e.g., fibrinolytic drugs), and hypothyroidism. The current incidence of rhabdomyolysis is approximately one case per 10,000 person-years [13]. Rhabdomyolysis is also associated with renal failure and a high mortality rate reported as 0.3 per 100,000 person-years [8].

2.3 New-onset diabetes

The first indication that statins may precipitate new-onset diabetes was reported in 2008 (JUPITER study), when the beneficial effects of rosuvastatin were evaluated in people with elevated high-sensitivity C-reactive protein (hs-CRP) levels but without hyperlipidemia [14]. This study indicated that rosuvastatin did not cause a significant increase in myopathy or cancer but did cause a higher incidence of diabetes, possibly because inclusion required elevated hs-CRP, a marker for insulin resistance.

Stains, especially lipophilic stains, can raise blood glucose levels and cause diabetes through a variety of potential mechanisms, such as inhibiting the synthesis of ATP and coenzyme Q10, increasing the uptake of plasma-derived LDL-C, decreasing the expression of glucose transporter 4 and the L-type calcium channel, and causing β-cell inflammation, oxidation, and apoptosis. However, the exact mechanism by which statins increase the risk of diabetes is unclear. It has been suggested that the inhibition of HMGCoAR may be a key mechanism for the increased risk of diabetes induced by statins [15].

Although statin does have an association with an increased risk of type 2 diabetes, not all statins display this effect. However, based on the current evidence, the cardiovascular protective benefits of statins still outweigh this risk, and it is necessary to strengthen the prevention and treatment of diabetes. Blood glucose should be monitored and adjusted in a timely manner when statins are used in clinics.

2.4 Cognitive function

It is unclear whether statins affect cognitive function in a positive or negative manner. Although there is evidence supporting the neuroprotective effects of statins, there are also reports suggesting that statins may adversely affect cognitive function [16]. Several epidemiological studies and meta-analyses reported a lower risk of dementia in statin users. Other studies also documented the reversible cognitive impairment, manifesting in restlessness, mental confusion, and short-term memory loss after beginning statin therapy or increasing the dosage [17, 18].

Poor cognitive performance has also been correlated with decreased serum cholesterol [18]. Higher doses of lipophilic statins, which can successfully pass the blood–brain barrier (BBB) and lower cholesterol levels in the central nervous system (CNS), are thought to cause negative consequences when exposed to the CNS [1]. In this context, cholesterol has many important functions in the brain, including myelin sheath formation, neuron signaling processes, and mitochondrial function [19]. In perspective, the FDA has put a neurological side effect warning label on statins due to the possibility of increased dementia, mild cognitive impairment, or cognitive performance decline [20]. However, several systematic reviews have shown that the effects

of statins on cognitive dysfunction are negligible [21]. Thus, the cognitive effects of statins have not been fully elucidated.

2.5 Renal function

In patients with chronic kidney disease (CKD), statins have generally been universally acknowledged for their renal protective functions. So they are strongly recommended for non-dialysis patients in stage 3 CKD [22]. A network meta-analysis of 43 randomized controlled trials (RCTs) revealed that statins slowed the progressive decline of estimated glomerular filtration rate (eGFR) and reduced proteinuria in CKD patients [22]. Although statins generally have a positive effect on renal function in these patients, there still exist some adverse effects concerning the kidneys that require special attention. Since myoglobin is released from muscle tissues, renal failure can be typically induced by rhabdomyolysis, one statin-associated muscle symptoms mentioned above. Myoglobin can then lead to renal dysfunction through inducting renal vasoconstriction, intratubular cast formation, and tubular cell toxic effects mediated by ROS [23]. This adverse effect, however, is exceedingly uncommon in the general population. There is also an increased risk of hospital admission in patients with acute kidney injury taking high-intensity statins compared with low-intensity statins [24], with the strongest effect observed within the first 4 months of starting statin therapy [25]. This suggests a dose-dependent injury of statin therapy on renal function. There are also reports indicating that kidney damage caused by statins may inhibit receptor-mediated endocytosis, hinder the reabsorption of protein by the proximal tubule, and lead to proteinuria [26]. Although there is a report that the relative hazard ratio for acute kidney injury in patients using statins for more than 1 year versus nonusers was 1.5 [25], clinical studies such as CARE debunked this notion. In reviewing the previous literature, no strong enough evidence-based medical evidence was found that statins affect the kidney and urinary system by inducing proteinuria. Overall, available studies do not suggest that statins deleteriously affect renal function.

2.6 Interstitial lung disease

The first case of interstitial lung disease (ILD) linked to statin was reported in 1995 [27]. Statin-induced ILD is a potential, recently identified side effect of statin medication, according to the FDA-AER. However, the mechanism of lung damage is not known [28]. In contrast, a cohort [29] and case-control study [30] both found no association between statin use and ILD. The only large study linking statin use and ILD is COPDGene [31]. In accordance with the COPDGene study, statin use is associated with ILA among smokers and promotes bleomycin-induced lung inflammation and fibrosis in mice via a mechanism involving heightened NLRP3 inflammasome activation. How statins can exacerbate ILD is unknown, but the effects on lipid metabolism via phospholipidosis [32] and the immune system via cytokine enhancement [33] have been considered possible mechanisms. Nevertheless, the relationship between statins and ILD is largely anecdotal and speculative. It is suggested that such adverse reactions should be treated in time and relevant cases should be recorded.

2.7 Other reactions

Studies have shown that the common adverse reactions of statins also include pancytopenia, stomatitis, irritability, increased blood urea, decreased blood pressure,

decreased white blood cell count, mental distress, cystitis, and so on. However, the study has limitations. One of the limitations with these conclusions is that the method used relies on a spontaneous adverse reaction monitoring system. The causal relationship between adverse reactions and drugs only depends on the judgment of the reporter, and the number and quality of reports are difficult to control. Therefore, the interpretation of data mining results should be cautious, and comprehensive judgment should be combined with evidence-based medical evidence. More crucially, a new study suggests the existence of potential for enhancement in how medical facilities consistently capture statin-associated side effects (SASEs) [34].

3. Conclusion and perspectives

In recent years, with the increasing evidence of preclinical and clinical research, the concept of using statins in patients has been constantly changing. Statins are widely used in clinical practice and have high safety in practical applications, but there are still adverse reactions in liver injury, renal function, myopathy, new-onset diabetes, cognitive impairment, and other aspects, which affect the treatment effect and the quality of life in patients. Therefore, we should attach great importance to the occurrence of adverse reactions in the process of actual clinical drug use, clarify their characteristics, and take more targeted prevention and treatment measures to ensure that the effect of clinical drug use can be improved. At present, research on statins in patients is still hot. It is believed that shortly, the role of statins will be clearer, and the use of drugs will be more standardized.

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Statins are now the most widely used agents in patients with dyslipidemia, atherosclerosis and cardiovascular disease. However, their effects and mechanisms have not been fully investigated, and research continues to reveal new aspects of statins. The up-to-date content of this book will be useful to physicians in cardiovascular disease and geriatrics, and to academics conducting research on statins. It includes novel perspectives leading to new insights and advancing existing knowledge on statins.

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