Review Article

Statin as Pleiotropic Modifier of Vascular Oxidative Stress, Inflammation and Thrombogenesis

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Abstract

Dyslipidemia causes progressive atherosclerosis in the vascular system which ultimately leads to cardiovascular diseases (CVD). CVD is the leading cause of mortality and morbidity worldwide. Atherosclerosis is the main cause of underlying CVD. It was previously considered as lipid storage disease but now growing evidence indicates that there is in addition, heightened oxidative stress characterized by lipid oxidation and inflammation of the blood vessels which contributes to the pathophysiology of atherogenesis. Event of atherosclerosis is initiated by oxidative stress through the production of reactive oxygen species as well as endothelial dysfunction. Several pro-inflammatory and anti-inflammatory cytokines and proteins are also involved in this process which causes activation of adhesion molecules that promote leukocyte rolling and infiltration into the sub-endothelial space. Platelet hyperactivity induced by dyslipidemia is associated with a high incidence of thrombogenic complications due to increase formation of thromboxane A_2 (TxA_2). The most promising available therapy for treating hyperlipidemia is the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors which are popularly known as statins. In addition to lipid lowering effect statins possess anti-oxidative, anti-inflammatory and anti-thrombogenic effects and this effect is independent of the effect on cholesterol levels in blood vessels or circulation. As oxidative stress, inflammation and thrombogenic property play important role in the development of atherosclerosis, it is being expected that treatment modalities targeting oxidative stress, inflammation and reducing proinflammatory cytokines and CRP levels could be a potential additional strategy in the primary and secondary prevention of CVD.

Keywords: *Atherosclerosis, Cardiovascular disease, Oxidative stress, Inflammation, Thrombogenesis, Statins.*

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Introduction:

Dyslipidemia or elevated blood lipid concentrations is a pathological condition which ultimately leads to atherosclerosis. Atherosclerosis is a chronic disease of arterial wall and may give rise to myocardial infarction (MI), ischemic stroke and peripheral vascular disease (PVD) as its aftermath.[1] HMG Co-A reductase inhibitor (statins) are quite effective in treating dyslipidemia and therefore widely used for prevention and treatment of cardiovascular diseases (CVD). Studies have suggested that statins reduce the incidence of CVD in dyslipidemic patients also by reduction of oxidative stress, inflammation and thrombus formation. Apart from their lipid-lowering action, statins exert some cholesterol-independent effects, also known as "pleiotropic effects" of statin.[2]

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Incidence of dyslipidemia in and around the world

In 2008 the global prevalence of raised total cholesterol among adults (≥5.0 mmol/l) was 39% (37% for males and 40% for females). The prevalence of elevated total cholesterol was

highest in the WHO Region of Europe (54% for both sexes), followed by the WHO Region of the USA (48% for both sexes). The WHO African Region and the WHO South East Asian Region showed the lowest percentages (22.6% for AFR and 29.0% for SEAR).[3] One recent study in Bangladesh involving 3201 individuals found rising trend of dyslipidemia in sub-urban population where prevalence of dyslipidemia was 16.6% in general, 22.2% in males and 15.9% in females. Total cholesterol (TC) was high (>240 mg/dl) in 16.9%, low density lipoprotein cholesterol (LDL-C) was high (>160 mg/dl) in 15.7%, high density lipoprotein cholesterol (HDL-C) was low (<40 mg/dl) in 8.8%, and triglyceride (TG) was high (>200 mg/dl) in 17.8% and very high (>350 mg/dl) in 2.0%.[4]

Cellular components of atherosclerosis

Atherosclerosis is a chronic, inflammatory disease of medium-sized and large arteries where endothelial cells, leukocytes and intimal smooth muscle cells are the major components affected. Elevated plasma cholesterol, hypertension, diabetes, smoking, male gender are the important risk factors whereas exercise, HDL and its major apolipoprotein, apoA-1 are considered as protective factors against atherogenic transformation of the arteries.[5] Atherosclerotic lesions are composed of three major components. The first is the cellular component comprised predominately of smooth muscle cells and macrophages. The second component is the connective tissue matrix and extracellular lipid. The third component is intracellular lipid that accumulates within macrophages, thereby converting them into foam cells.[6]

Endothelial dysfunction and atherosclerosis

Endothelial dysfunction is the early feature of initiation of atherosclerosis. Function of endothelium is regulated by balance between vasodilator nitric oxide (NO) and vasoconstrictor substances (endothelin, angiotensin II). NO is produced by endothelial cell by endothelial nitric oxide synthase (eNOS). Along with vasodilator effect NO inhibits leukocytes infiltration, smooth muscle cells (SMC) proliferation and platelet aggregation. It also prevents oxidative modification of LDL to oxidized LDL (ox-LDL).[7]

Role of oxidative stress in producing atherosclerosis

Under normal physiological condition a balance is maintained between generation of oxygen free radicals and antioxidant defense systems. Impairment in this equilibrium provokes a situation of oxidative stress.[8] Oxidative stress is a condition where there occurs increased production of reactive oxygen species (ROS). ROS also termed as "oxygen-derived species" or "oxidants" and are produced as intermediates in the pathway of reduction-oxidation reactions. For the development of atherosclerosis ROS plays an important role. ROS are produced by all vascular cells due to excess production of oxidants and decreased nitric oxide (NO) bioavailability. It includes two major groups: free radicals (e.g., superoxide [$\cdot O_2^-$], hydroxyl [OH·], nitric oxide [NO·]) and nonradical derivatives of O_2 (e.g., H_2O_2, $ONOO^-$).[9] Elevated production of ROS by endothelial and smooth muscle cells causes oxidative modification of LDL to ox-LDL. Ox-LDL can damage vascular endothelium and induce adhesion molecule expression and consequent monocyte accumulation which is the early initiating feature of a atherogenic lesions.[10]

Inflammation and atherosclerosis resulting in plaque formation

Inflammation plays a central role in all phases of atherosclerotic process. Endothelial dysfunction occurs due to damage of endothelium by circulating mediators and physical forces. High concentration of blood LDL level favours monocytes to ingest lipoprotein to become macrophages. Macrophages engulf ox-LDL to form foam cells. There is increase production of pro-inflammatory cytokines (IL-6, 8,18) and increased expression of vascular cell adhesion molecules-1 (VCAM-1), monocyte chemoatractant protein-1 (MCP-1). Under all of these influences monocytes, T-lymphocytes and SMC (smooth muscle cells) migrates into the subendothelial space.[11] All these cells and foam cells coalesce to form fatty streak. Foam cells secrete some growth factors which proliferates and migrates SMCs from media to neointima. SMCs proliferation along with continuous influx and proliferation of monocytes converts fatty

streak into fibrous plaque. Further calcification and fibrosis yeild a fibrous cap surrounding a lipid rich core.[12]

Dyslipidaemia and vascular thrombosis and their consequences

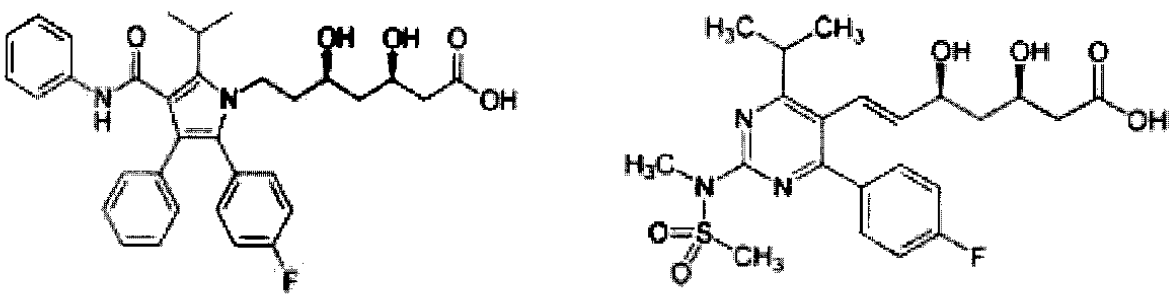
Thrombosis in vascular wall leading to platelet aggregation, thrombus formation, vascular occlusion which ultimately leads to possible MI or ischaemic stroke. High levels of LDL-cholesterol, hypertension, smoking are important risk factor for the development vascular thrombosis.[13] Platelet activity has been enhanced in the presence of high LDL cholesterol levels. There is a strong relationship between the cholesterol levels to platelet reactivity and thromboxane production.[14,15] Platelet is activated in hyperlipidemic patients due to increase formation of thromboxane A_2 (TxA_2), a potent platelet aggregation activator.[16] When platelet stimulated there is an enhanced release of ADP and ATP. ADP and ATP via P2Y-receptors on platelets leads to recruit further platelets to form a thrombus and trigger the release of thrombin as a pro-inflammatory mediator.[17]

Pharmacology of statin

Among many cholesterol-lowering agents statins or HMG-CoA reductase inhibitors are widely applied in cardiovascular and coronary heart diseases. Besides its lipid lowering action statins exhibits significant contribution on improvement of endothelial dysfunction, inhibition of oxidative stress, vascular inflammation and stabilization of atherosclerotic plaque which all together known as pleiotropic effects of statin.[18] Among commonly used statins lovastatin, pravastatin and simvastatin are fungal derived inhibitors of HMG-CoA reductase, where atorvastatin, ûuvastatin, pitavastatin and rosuvastatin are fully synthetic. Statins inhibit conversion of HMG-CoA to mevalonate which is rate limitimg step of hepatocyte cholesterol synthesis.[19] Several drugs are included within the statin group, among them atorvastatin and now-a-days rosuvastatin is widely used. Both atorvastatin and rosuvastatin form an additional hydrogen bond with the Ser565 residue in the enzyme and the carbonyl oxygen of atorvastatin or the sulfone oxygen of rosuvastatin. Rosuvastatin exhibits an additional and unique polar interaction between its sulfone group and the enzyme Arg568 side chain in the enzyme. These explains that rosuvastatin has the greatest number of binding interactions with the enzyme active site. Due to having an additional interaction with the enzyme and differences in the number and types of bonds between the statins which is absent in other synthetic statins may explain the relatively greater efficacy of atorvastatin and rosuvastatin.[20]

Antioxidative role of statin

Statins effectively reduce cardiovascular events of patients with hyperlipidemia. By inhibiting synthesis of mevalonate statins inhibit isoprenoid intermediates such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) which leads to an inhibition of isoprenylation of small GTPases such as Ras, Rho. Statins increase eNOS expression and increase NO bioavailability by inhibiting isoprenylation of Rho GTPases which improve endothelial function in addition to cholesterol reduction.[21] It also modulates



Atorvastatin Rosuvastatin

Fig.-1: *Chemical structure of atorvastatin and rosuvastatin.*[19]

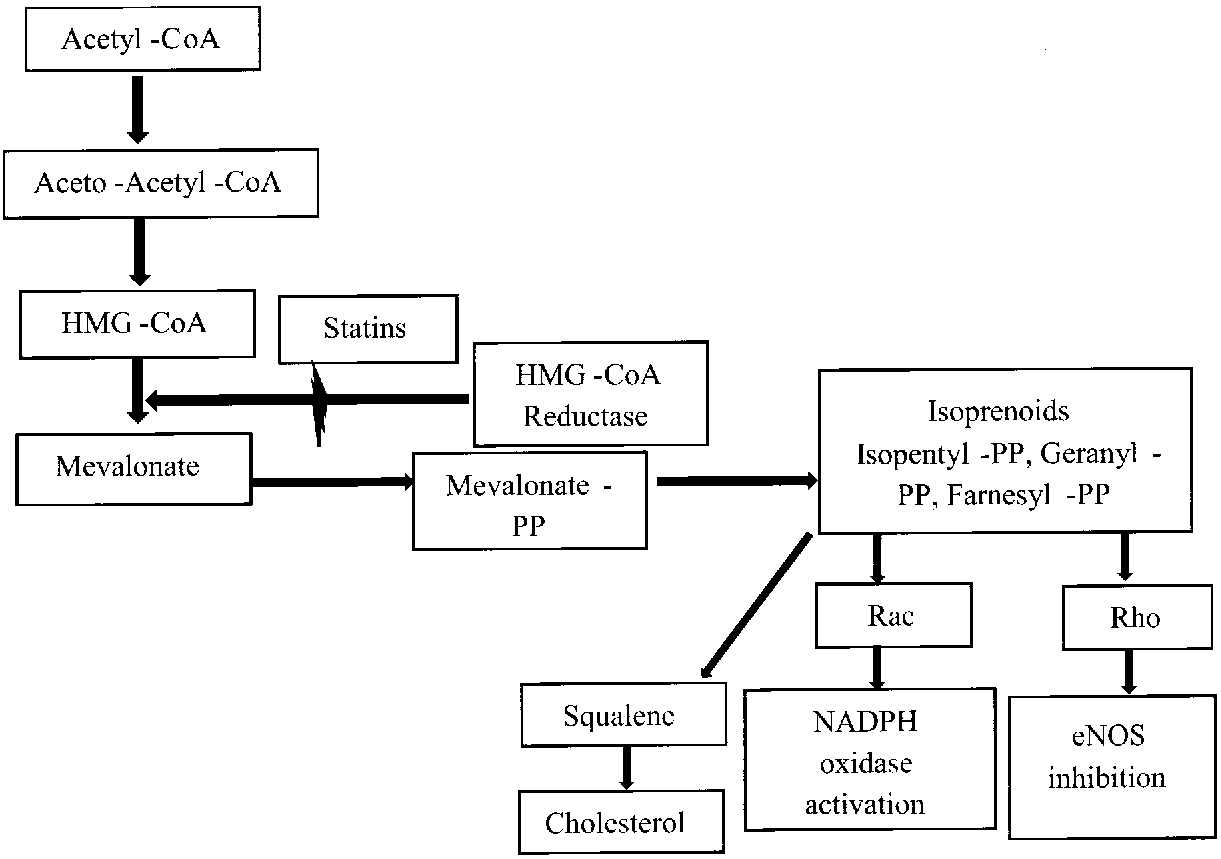


Fig.-2: *Cholesterol biosynthesis pathway and the effects of statins. Inhibition of HMG-CoA reductase by statins decreases isoprenoid intermediates such as farnesyl-PP and geranylgeranyl-PP, which leads to an inhibition of isoprenylation of small GTPases such as Ras, Rho, Rab, and Rap. Among the Rho GTPases are RhoA, Rac1, and Cdc42. CoA indicates coenzyme A; PP, pyrophosphate.*[21]

NADPH-oxidase enzyme activity by reducing translocation of Rac-1. NADPH-oxidase a multicomponent enzyme and act as an important source for production of ROS.[22,23]

There are several anti-oxidative enzymes which are involved in the elimination process of ROS and preserve the balance of ROS availability within vascular cells. Statins increase expression of the radical-scavenging enzymes manganese SOD (mnSOD), extracellular SOD (ecSOD), copper-zinc SOD (czSOD), glutathione peroxidase (GPx) or CAT.[24] Atorvastatin is more effective in inhibiting hydroperoxide formation and increasing activity of catalase.[22,25] This effect of the statins perhaps aids in the elimination of ROS from the body.

Anti-inflammatory role of statins

Ox-LDL and inflammatory cytokines (IL-6 and TNF-α) induces expression of leukocytes adhesion molecules ICAM-1/CD54 and its ligand lymphocyte function-associated antigen-1 (LFA-1 or $CD11_a$/CD18) expression providing essential step in atherogenesis.[26] Statins also reduces MCP-1 and other proinflammatory cytokines such as IL-β, 6 and TNF-α.[27] One study shows treatment with simvastatin causes significant reduction of the expression of ICAM-1 and its ligand LFA-1 on monocytes of hypercholesterolemic patients. This loss of adhesive function may contribute to the anti-inflammatory effects of statin.[28]

Elevated serum level of CRP is nonspecific but sensitive marker of the acute inflammatory response. Study shows that CRP can induce adhesion molecule expression (ICAM-1, VCAM-1, and E-selectin) by human endothelial cells at concentrations ≥5 μg/mL. It indicates a significant pro-inflammatory effect of CRP in endothetial cells.[29] A randomized, open-labeled trial on 100 patients with acute coronary

syndrome was carried out to compare anti-inflammatory effect of atorvastatin and rosuvastatin. After 4 weeks treatment with atorvastatin 40 mg and rosuvastatin 20 mg daily CRP level significantly decreased (35% in atorvastatin group and 44% in rosuvastatin group, both P< 0.001). Both drugs show favourable effect on lipid profile with a significant decrease in total cholesterol, LDL-C and TG but very mild decline of HDL cholesterol.[30]

Antithrombotic function of statin

Incidence of atherosclerosis and its thrombotic complications are highly associated hypercholesterolemia.[16] Statins suppress expression of TF (serve as cofactor for plasma factor VII) which plays role in initiation of extrinsic coagulation pathway. Inhibition of geranylgeranylated protein involved in TF biosynthesis may be the possible mechanism.[31] Statins also reduce platelet TxA_2 via down regulation of plospholipase A_2 and inhibit platelet aggregation. Atorvastatin inhibits arachidonic acid (AA) induced PLA_2 phosphorylation and TxB_2 and shows antiplatelet effect in patients with hypercholesterolemia.[32]

Along with inhibition of TF expression and TxA_2 level reduction antiplatelet effect of statin may be mediated by its effect on PECAM-1 signaling. Study shows simvastatin increase levels of tyrosine phosphorylation of PECAM-1. This Activation of PECAM-1 signaling results diminution of phosphoinositol 3-kinase (PI3K) signaling which ultimately inhibit platelet activation.[33]

Conclusion:

It appears that the statins exert multifactorial beneficial effects "pleiotropic effects" in addition to their anti-lipid action. Thus the pleiotropic effects of the statins together with their cholesterol synthesis inhibiting action probably create a protective barrier against vascular atherosclerosis and clears free radicals from the body. This may ultimately reduce incidences of cardiovascular accidents such as MI or stroke.

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