



Pleiotropic effects of statins in stroke prevention

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ABSTRACT

Cardiovascular disease is the leading cause of death and disability, and contributes substantially to healthcare budgets. The lipid-lowering drugs, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor or statins, reducing mortality and cardiovascular morbidity in patients with established cardiovascular disease. Statins therefore have a place in the secondary prevention of cardiovascular disease. Recent experimental and clinical studies suggest that statins may exert vascular protective effect beyond cholesterol reduction. The cholesterol-independent or "pleiotropic" effects of statin include the upregulation and activation of endothelial nitric acid synthase (eNOS) that can increase nitric oxide (NO) production. Augmentation of NO production increases cerebral blood flow, which can lead to neuroprotection during brain ischaemia. By inhibiting mevalonate synthesis, statins prevent the formation of several isoprenoids (including farnesylpyrophosphate and geranylgeranylpyrophosphate). Inhibiting geranylgeranylation of RhoA small G proteins increases the stability of eNOS mRNA through the remodeling of endothelial actin microfilaments. Moreover, statins directly increase eNOS activity within minutes by activating the pathway involving phosphoinositide 3-kinase and protein kinase B. In the secondary prevention of stroke, the use of statins reduces the incidence of either recurrent stroke or other major vascular events and treatment should be initiated soon after the event. The use of statins does not increase hemorrhagic stroke or cancer and may also favor atherosclerotic plaque regression.

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INTRODUCTION

According to data from Heart Disease and Stroke Statistic Update in 2009, each year 795.000 stroke cases are reported in the United States, consisting of 610.000 cases of first stroke

events and 185.000 cases of repeat stroke. Stroke is also reported to be the third major cause of death after heart disease and cancer. Total mortality in 2005 in the United States is reported to amount to 242.000 cases, and in 143.579 cases the cause of death is stroke.⁽¹⁾

To date there is no effective pharmacological treatment for stroke. The development of drugs capable of preventing stroke events will play an important role because of the high mortality and disability resulting from this disorder, which not only imposes an economic burden but also has an impact by lowering the quality of life of the patient.

The discovery of statins in 1976 by Akira Endo occurred fortuitously in the course of a search for new antibiotic compounds.⁽²⁾ The discovery of statins was a major advance in prevention and treatment of ischemic stroke, similar to the discovery of antiplatelet and antithrombotic drugs, with the objective of maintaining and restoring cerebral blood flow.

To date it is debatable whether increased serum cholesterol level is a risk factor for stroke events, and whether statins have a preventive effect on stroke events. The Cholesterol Treatment Trialists Collaborators⁽³⁾ in their meta-analysis comprising 90,056 patients showed that the use of statins significantly resulted in a proportional reduction of first stroke events of 17% for each 1 mmol/L reduction of low-density lipoprotein (LDL) concentration. However, it is unclear whether the results of this meta-analysis are caused by the effects of statins in lowering LDL cholesterol levels or by the (*Greek*: “pleio” or many, and “tropos” manner) pleiotropic effects of statins. The Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study,⁽⁴⁾ which had the objective of evaluating the secondary preventive effects of statins on stroke, demonstrated that atorvastatin reduced the risk of repeated cerebrovascular events in patients without a history of coronary heart disease who had recently experienced a stroke or transient ischemic attack (TIA).

The effects of statins that are independent of their blood cholesterol-reducing effects are also called their pleiotropic effects. These are

apparently related to the ability of statins to improve plaque stability, reduce the number of inflammatory cells in plaques, restore endothelial function, inhibit platelet function, increase fibrinolytic activity, cerebral blood flow and nitric oxide (NO) levels. Due to their pleiotropic effects, statins have been claimed to possess neuroprotective effects in the prevention of ischemic stroke.^(5,6)

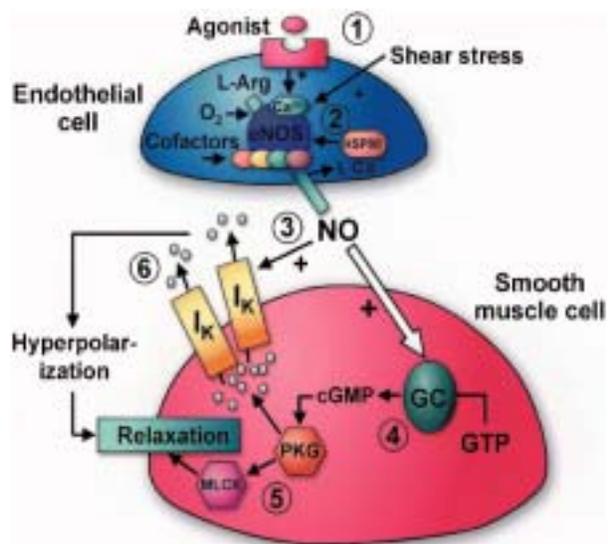


Figure 1. Biosynthesis of NO and effects of NO on blood vessels⁽⁸⁾

The biosynthesis of NO is mainly performed by an isoform of eNOS that is dependent on the presence of Ca^{2+} . This process is triggered by binding of agonists or by fluid shear stress (1) and is facilitated by several cofactors and the HSP90 heat-shock protein. Conversion by eNOS of L-arginine (L-Arg) to NO (2) gives rise to L-citrulline (L-cit) as a by-product. Subsequently NO diffuses into the neighboring smooth muscle cells (3) where it activates the effector enzyme guanylate cyclase (GC). GC (4) then converts guanosine triphosphate (GTP) into the second messenger cyclic guanosine monophosphate (cGMP), which in turn activates protein kinase G (PKG) (5) resulting in modulation of myosin light chain kinase and smooth muscle relaxation. PKG also modulates the activity of potassium channels (hyperpolarization) and causes relaxation. NO itself can also directly modulate potassium channels (independently of cGMP).

The present review discusses the pleiotropic effects of statins in the prevention of ischemic stroke in connection with endothelial nitric oxide synthase (eNOS). In addition, this paper aims to find supporting evidence for a rationalized utilization of statins in ischemic stroke prevention.

NO, eNOS and vascular disorders

eNOS (endothelial nitric oxide synthase) is an enzyme for the production of nitric oxide (NO) by vascular endothelium.⁽⁷⁾ NO is produced by eNOS through oxidative conversion of L-arginine to L-citrulline. Activation of eNOS occurs through specific phosphorylation at Ser1177 by protein kinase B (PKB/Akt), which also mediates NO synthesis induced by fluid shear stress. After synthesis by the vascular endothelium, NO diffuses into the neighboring cells and activates soluble guanylate cyclase. This process subsequently mediates various beneficial effects of NO.⁽⁷⁾

In vascular smooth muscle, NO is a potent vasodilator and regulates regional blood flow. Figure 1 presents a diagram of biosynthesis of NO and its effects on blood vessels.⁽⁸⁾

In addition to the vasodilator effects of NO, other beneficial effects of NO are its antithrombotic, anti-inflammatory, and antiproliferative effects. On the other hand, loss of NO leads to impaired vascular relaxation, platelet aggregation, increased proliferation of

vascular smooth muscle, increased leukocyte adhesion to the endothelium, and raised blood pressure. Therefore it may be stated that endothelial NO acts as a protector of the vascular wall.⁽⁷⁾

Currently three isoforms of nitric oxide synthase (NOS) have been identified in mammals,⁽⁹⁾ each of these NOS isoforms being encoded by a different gene and showing differences in location and function, as presented in Table 1.

In the brain, besides eNOS, other NOS isoforms, namely neuronal NOS (nNOS) and inducible NOS (iNOS), also play a role during cerebral ischemia. Like eNOS, nNOS is constitutively expressed and is Ca²⁺/calmodulin-dependent. Macrophage NOS ('immunologic' NOS or iNOS) is induced by selective immunologic stimuli and is Ca²⁺-independent.⁽⁷⁾

The NO produced by eNOS and nNOS is essential for the regulation of cerebral blood flow,^(7,9) and eNOS is also said to play a role in reducing the extent of an infarction.⁽⁷⁾ Furthermore, NO from nNOS also functions as a neurotransmitter and is involved in synaptic plasticity, modulation of neuroendocrine function, and behavioral activity.⁽⁹⁾ In pathological conditions, such as cerebral ischemia, NO is produced in large quantities in the brain as a result of induced expression of iNOS. Induction of iNOS expression occurs as a result of increased transcription of the iNOS

Table 1. Differences of NOS isoforms⁽¹⁰⁾

Name	Gene	Location	Function
Neuronal NOS (nNOS/NOS1)	NOS1	Nervous system	Cellular communication
Inducible NOS (iNOS/NOS2)	NOS2A,2B,2C	Immune system Cardiovascular system	Immunity to pathogens
Endothelial NOS (eNOS/NOS3/cNOS)	NOS3	Endothelium	Vasodilation

gene in response to locally produced inflammatory cytokines. The ischemia induced by overproduction of NO is related to glutamatergic activation mediated by increased intracellular Ca^{2+} concentrations, which causes calmodulin-dependent upregulation of nNOS and eNOS activity.⁽⁹⁾

The augmented NO production in the early ischemic phases occurs as a result of increased eNOS and nNOS activity, which is of short duration (~1 hour) and rapidly decreases within a few hours, both in transient and permanent ischemia. This process is subsequently followed by the second stage of large-scale NO production as a result of induced expression of iNOS, which is started several hours after the initial ischemic phase and persists up to 4-7 days. NO production by iNOS apparently contributes secondarily to cerebral damage that occurs in the final stages.⁽⁹⁾

Depending on the cellular source and evolutionary stage of the ischemic process, NO may be protective or destructive. The double role of NO in cerebral ischemia forms the basis for a selective therapeutic approach aimed at inhibiting nNOS and iNOS, while increasing eNOS.⁽⁷⁾

STATINS AND eNOS

Statins are a class of drugs that are known to be capable of increasing eNOS levels. The principal mechanism of action of statins is reduction of cholesterol levels. This effect is mediated by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors in the liver, which are necessary for cholesterol biosynthesis.

Around 60-70% of serum cholesterol is synthesized in the liver, with HMG-CoA reductase playing an essential role in the cholesterol biosynthetic pathway. Oxidized low-density lipoprotein (LDL) has been known to be capable of inhibiting the expression of eNOS that

is bound to caveolin-1 (an integral protein component of the cell membrane) within caveolae.⁽⁷⁾ Inhibition of HMG-CoA reductase by statins causes a dramatic reduction in circulating LDL cholesterol levels (Figure 2).⁽¹¹⁾ Additionally, reduction of LDL cholesterol leads to upregulation of LDL receptors, thus effecting increased LDL clearance. This effect is presumably due to the ability of statins to reduce the abundant amounts of caveolin-1.⁽⁷⁾

Besides their cholesterol-lowering effects, statins also exert cholesterol-independent or pleiotropic effects, as a result of the ability of statins in inhibiting the conversion of HMG-CoA to L-mevalonic acid. Inhibition of L-mevalonic acid synthesis by statin leads to inhibition of the synthesis of 'isoprenoid' intermediates such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP). Isoprenoids function as lipid attachment sites for intracellular signal molecules. Statin causes inhibition of isoprenylation required for modification of small G proteins (GTPases), one of which being the Rho A protein, to enable them to occupy the appropriate locations at the membrane and thus perform their functions. Isoprenylation inhibition by statin prevents downregulation of eNOS expression, and maintains eNOS activity in conditions where downregulation of eNOS expression occurs, such as hypoxia (e.g. ischemic stroke) and the presence of oxidized LDL in the blood. This process occurs through inhibition of the Rho kinase activator (Rho A), leading to post-translation stabilization of eNOS mRNA and prevention of downregulation of eNOS expression.^(11,12)

Currently there are 8 subfamilies of GTPases that have been identified in mammals. In performing its function, GTPases undergoes cyclical changes from the GDP-bound (inactive) form to the GTP-bound (active) form, and vice versa.⁽¹¹⁾ Of the existing 8 subfamilies of GTPases, only the Ras and Rho GTPases have

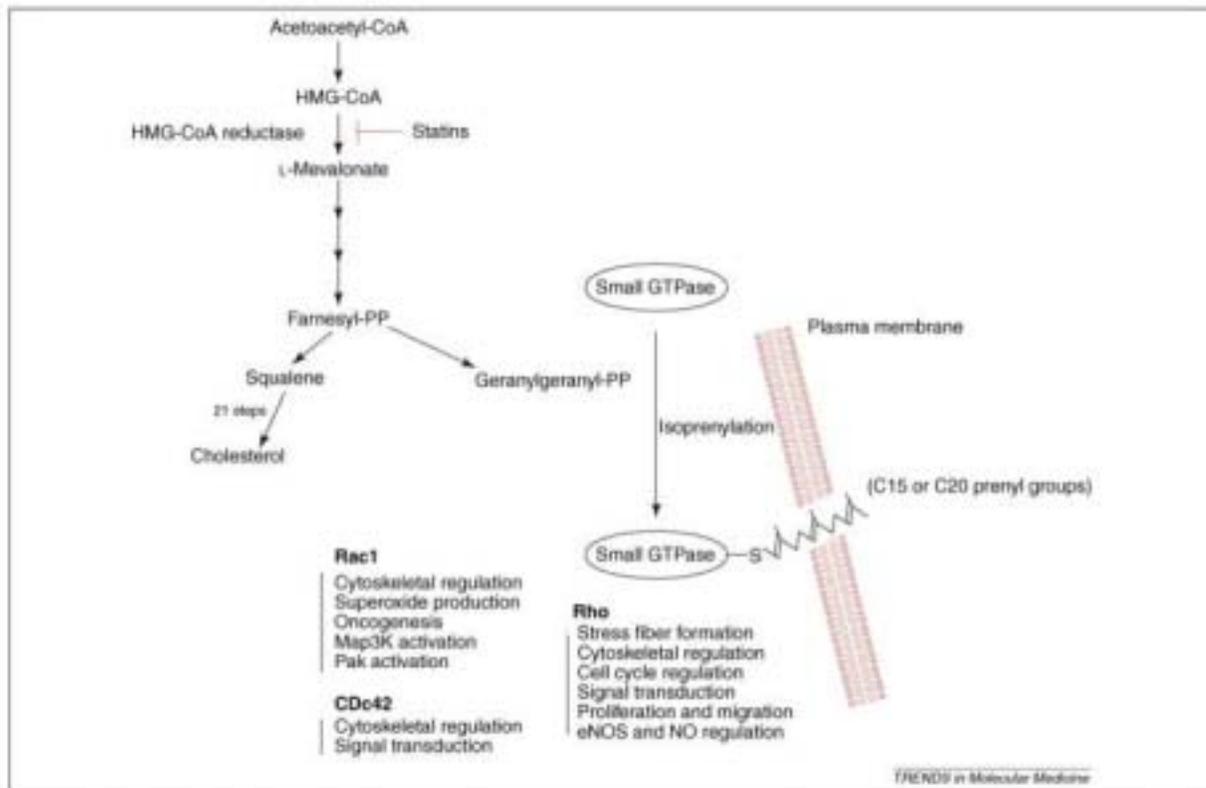


Figure 2. Isoprenoids and statins⁽¹¹⁾

Diagram of cholesterol biosynthetic pathway and inhibition of HMG-CoA-reductase by statins. Inhibition of isoprenylation by statin results in modulation of various cellular functions

merited special attention, as they effect transduction of extracellular stimuli into multiple intracellular signal transmission.⁽¹¹⁾

The Ras family plays an important role in signal transduction, and in cellular proliferation and malignant transformation. Representatives of the Rho GTPases comprising RhoA, Rac and Cdc42, have specific functions in cellular shape, motility, secretion, and proliferation. The biological effects of the Rho GTPase family is presented in Figure 2. Inhibition of geranylgeranylation of RhoA GTPase by statin enhances stability of eNOS mRNA through remodelling of endothelial actin microfilaments.⁽¹¹⁾

In addition to inhibition of isoprenoid synthesis, statin directly enhances eNOS activity

by activation of the pathway involving phosphoinositide 3-kinase (PI3K) and PKB/Akt.⁽¹³⁾

EVIDENCE FROM ANIMAL MODEL STUDIES

Animal model studies with the objective of finding evidence for the neuroprotective effects of statins and their underlying mechanisms have been conducted. For inducing cerebral infarction in the experimental animals (rats), permanent occlusion of the middle cerebral artery was performed. The temporal progression of the resulting infarction was monitored by magnetic resonance imaging (MRI). Subsequently the

experimental animals were given simvastatin (20 mg/kg) on the third hour after occlusion. The study results showed that administration of simvastatin prevented increases in the volume of infarction within 24 hours and decreased the size of the infarction up to 46.6% within 48 hours after the intervention. On the basis of immunoreactivity assays of experimental animals receiving simvastatin, the neuroprotective effects of simvastatin paralleled the increase in eNOS.⁽¹⁴⁾

A study with the objective of determining the physiological relevance of regulation of eNOS expression by Rho kinase (ROCK) has been conducted. This study was performed by administration of fasudil (ROCK inhibitor) to rats in the 48-hour period prior to occlusion of the middle cerebral artery, followed by assessment of cerebral blood flow, extent of cerebral infarction, and neurological deficits. The results indicated that administration of ROCK inhibitors led to increased blood flow in the ischemic as well as the non-ischemic areas of the brain, reduction in size of infarction of up to 33%, and enhancement of neurological deficit score of up to 37%. These study results confirmed the premise that the neuroprotective effects due to ROCK inhibition was mediated by eNOS.⁽¹⁵⁾

STATINS AND SECONDARY PREVENTION OF STROKE

The Stroke Prevention by Aggressive Reduction of Cholesterol Level (SPARCL) is a study that for the first time investigates the effects of statins on cerebrovascular risk in patients without a history of coronary heart disease. This study is a multicenter, placebo-controlled double-blind randomized clinical trial. The atorvastatin dose used is 80 mg/day. Inclusion criteria in this study are patients with previous TIA or stroke, LDL levels between 100 mg/dL and 190 mg/dL, and without evidence of

coronary heart disease. The primary endpoint is the first occurrence of fatal and non-fatal (cerebrovascular) stroke, while the secondary endpoint is the first occurrence of a cardiovascular event.⁽¹⁶⁾

The number of subjects for this study was 4,731 persons with a history of stroke or TIA within the previous six months. After a 6-year follow up, there were 265 patients with fatal or non-fatal stroke in the group receiving atorvastatin, whereas in the control group there were 311 of these patients. There was a risk reduction of 16% of first occurrence of stroke in the group on atorvastatin (adjusted hazard ratio: 0.85, 95% CI: 0.71-0.99; number needed to treat: 46). For the secondary endpoint of this study, namely occurrence of stroke or TIA, there was a risk reduction of 23% (hazard ratio: 0.77, 95% CI: 0.67-0.88) with 375 events in the atorvastatin group and 476 events in the control group. Furthermore, there was a risk reduction of 35% in coronary events (hazard ratio: 0.65, 95% CI: 0.49-0.87).⁽⁴⁾

The Heart Protection Study⁽¹⁷⁾ investigated a total of 20,536 patients with coronary disease, other occlusive arterial disorders, or diabetes. Among the study subjects were 3,280 patients with stroke prior to randomization, of which 1,822 had stroke without evidence of CHD. The subjects were randomized for simvastatin 40 mg/day or placebo for 5 years. The results of this study showed a significantly reduced all-cause mortality, particularly mortality due to coronary disease. The number of stroke events was also reduced significantly up to 25% [444 stroke events in the simvastatin group (4.3%) versus 585 stroke events in the placebo group (5.5%); OR: 0.75; 95% CI: 0.66-0.79; $p < 0.0001$]. The reduction in stroke events was mainly due to ischemic stroke, while the number of hemorrhagic stroke events was similar in both groups.

Analysis of the HPS study results indicated that there was a reduction in major vascular events of 23%, but the interpretation of these results has been inconclusive. The reduction in the endpoint of this study included major coronary events, stroke, or revascularization, but the reduction was mainly for major coronary events and revascularization, as the number of events of repeat stroke was almost identical in both groups (10.4% simvastatin versus 10.5% placebo).⁽¹⁸⁾ The lack of an apparent effect of simvastatin in preventing repeat stroke may be due to the subgroup analysis performed or due to the small numbers of patients with repeat stroke, resulting in a decreased power for detection of differences.⁽¹⁹⁾

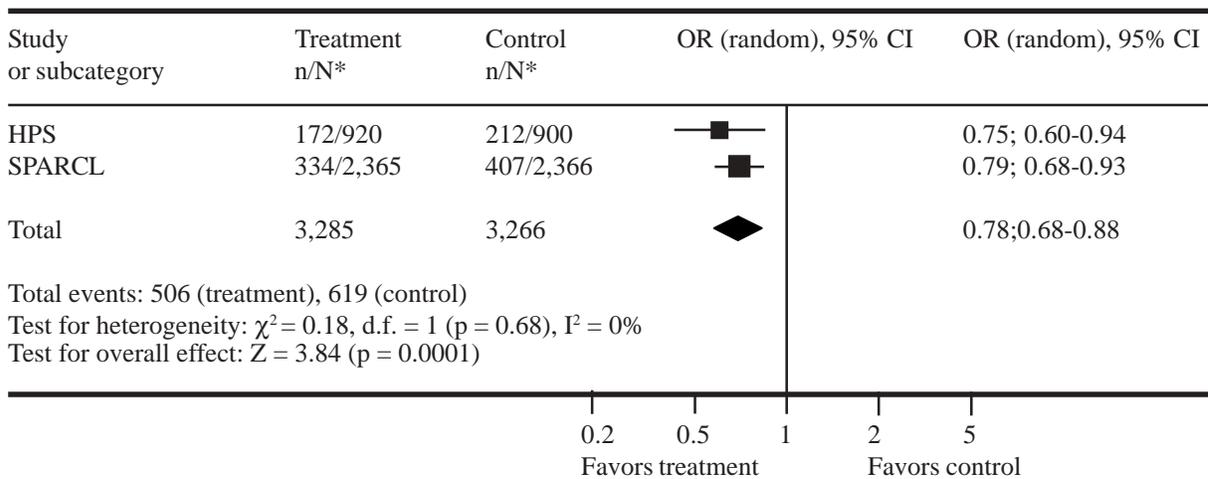
The mini meta-analysis conducted on the SPARCL and HPS studies of patients with a history of cerebrovascular disorders without CHD and with major vascular events and repeat stroke events is presented in Figures 3 and 4.⁽²⁰⁾ According to the results of the mini meta-

analysis on the SPARCL and HPS studies, when compared to placebo, statin shows a nearly non-significant difference for repeat stroke events (OR: 0.87, 95% CI: 0.75-1.01; p: 0.07), but a significant difference for major vascular events (OR: 0.78, 95% CI: 0.68-0.88; p: 0.0001).⁽²⁰⁾

CLINICAL PRACTICE

The American Heart Association/American Stroke Association⁽²¹⁾ has issued recommendations on stroke prevention in patients with a history of ischemic stroke or transient ischemic attack (TIA). The recommendations comprise two important points. Firstly, patients with ischemic stroke or TIA, having a high cholesterol level, coronary heart disease comorbidity, or evidence of atherosclerosis, should be managed according to the guidelines issued by the National Cholesterol Education Program (NCEP III),⁽²²⁾ involving lifestyle changes, dietary regulation, and use of medications (Class IA, evidence level A).

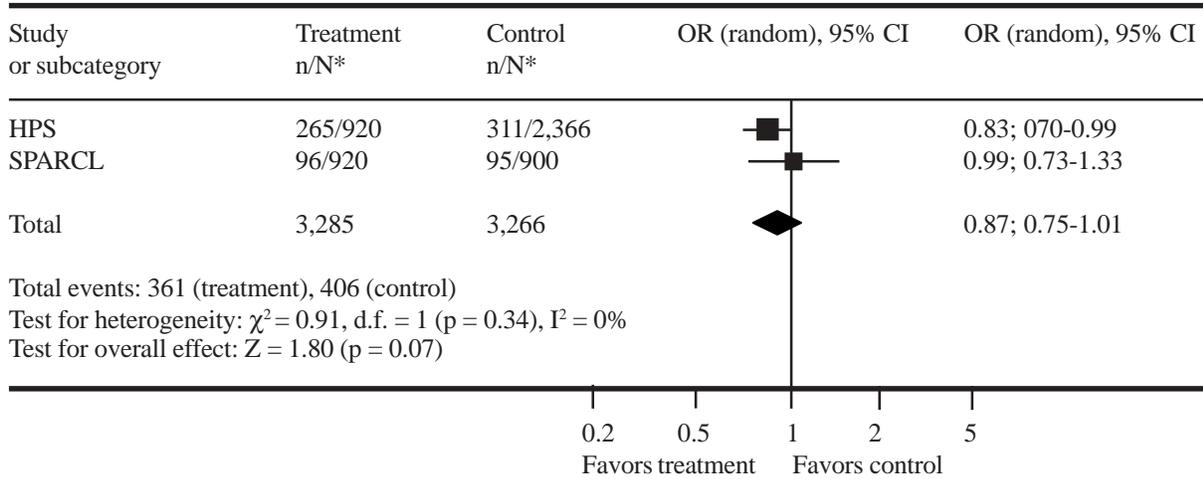
Comparison : Statins versus placebo
Outcome : Major vascular events



*n/N= number of subjects per event per total number of subjects

Figure 3. Effects of statin on major vascular events in patients with history of cardiovascular disease without CHD.⁽²⁰⁾

Comparison : Statins versus placebo
 Outcome : Recurrent



* n/N= number of subjects per event per total number of subjects

Figure 4. Effects of statin on repet stroke events in patients with history of cardiovascular disease without CHD.⁽²⁰⁾

Administration of statin is recommended, where the target of cholesterol reduction in patients with symptomatic CHD or atherosclerosis is LDL cholesterol <100 mg/dL, whilst for high risk patients with multiple risk factors (Class I, evidence level A) the target is LDL<70 mg/dL. Secondly, patients with ischemic stroke or TIA suspected of having been caused by atherosclerosis but without indications for statin usage (normal cholesterol level, without coronary heart disease comorbidity, or without evidence of atherosclerosis) are suitable candidates for statin therapy to reduce the risk of vascular events (Class IIa, evidence level B).

Based on the SPARCL study results, the SPARCL investigators suggest a modification of abovementioned clinical guidelines, by specifically stating that stroke and TIA shall be categorized as ‘coronary heart disease risk equivalent’ in connection with the indications for statin therapy.^(4,20)

CESSATION OF POST ISCHEMIC STROKE STATIN THERAPY AND CLINICAL OUTCOMES

The majority of post ischemic stroke patients are expected to derive benefits of long-term statin therapy. In clinical practice, however, the patient frequently discontinues the use of statins. A study conducted by Colivicchi et al⁽²³⁾ had the objective of assessing the impact of cessation of post ischemic stroke statin therapy, with a follow-up period of 12 months. The study subjects comprised 631 stroke patients without clinical evidence of coronary heart disease. The results of this study indicated that within a period of 12 months a total of 246 patients (38.9%) stopped statin therapy, with a mean period of statin therapy cessation of 48.6±54.9 days. During the follow-up period 116 patients died from cardiovascular (80.1%), and non-cardiovascular (6.9%) causes. This study also

showed that the patients dying during follow-up were commonly those who were elderly, had diabetes mellitus, and were obese. Discontinuation of statin therapy was said to be an independent predictor of all-cause mortality in the first year after cessation of statin therapy (hazard ratio: 2.78; 95% CI: 1.96-3.72; p: 0.003).

A study with similar objectives was performed by Liao et al⁽²⁴⁾ in Italy on 631 post ischemic stroke patients. Inclusion criteria were patients without clinical and laboratory evidence of coronary heart disease, or other cardiac disorders, who had stopped using statin. In the follow-up period 246 patients (38.9%) discontinued statin therapy, and 116 patients died within the first year after cessation of therapy. As in the previous study cessation of statin therapy was said to be an independent predictor of all-cause mortality within 12 months. It is to be regretted that there was no clear description of the causes of death, whether stroke or cardiovascular disease, as had been done in the study by Colivicchi et al.⁽²³⁾

The study findings mention the occurrence of a rebound effect due to statin withdrawal, where the clinical effects were more severe than the clinical effects of patients not on statin therapy. On the basis of the hypothesis that statin has pleiotropic effects through upregulation of eNOS, it is believed that cessation of statin therapy causes a reduction in NO release, due to rebound in the form of increased Rho GTPase levels, which inhibit eNOS expression.⁽²⁵⁾

CONCLUSIONS

Apart from the ability of statins to lower cholesterol levels, the pleiotropic effects of statins in enhancing the expression of eNOS result in the beneficial effects of these drugs when used in post acute ischemic stroke patients. On the basis of the results of a mini-metaanalysis

on the SPARCL and HPS studies, statins showed an almost non-significant difference for repeat stroke events, but a significant difference for major vascular events. The studies indicate that the discontinuation of statin therapy in post acute ischemic stroke patients leads to an almost threefold increase in mortality in the first year. It has even been stated that cessation of statin treatment is an independent predictor of all-cause mortality in the first year. Apparently the clinical recommendations issued by AHA/ASA regarding statin usage for stroke prevention in patients with a history of ischemic stroke or TIA are still applicable. The results of the SPARCL trial support the necessity of modifying the clinical guidelines, i.e. by specifically stating that stroke and TIA shall be categorized as 'coronary heart disease risk equivalent' in connection with indications for statin treatment. 

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