Cholesterol-lowering therapy and cell membranes. Stable plaque at the expense of unstable membranes?

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Abstract

Current guidelines encourage ambitious long term cholesterol lowering with statins, in order to decrease cardiovascular disease events. However, by regulating the biosynthesis of cholesterol we potentially change the form and function of every cell membrane from the head to the toe. As research into cell morphology and membrane function realises more dependencies upon cholesterol rich lipid membranes, our clinical understanding of long term inhibition of cholesterol biosynthesis is also changing. This review of non-cardiovascular research concerning such membrane effects raises important new issues concerning the clinical advantages and disadvantages of the long term use, and broadening criteria, of cholesterol reductions.

Key words: cholesterol, exocytosis, lipid, membrane, statin.

Introduction

The undoubted commercial success story in modern medicine has been the creation of that infamous household dietary and medical obsession: 'Cholesterol'. Over the past decade researchers have achieved new insight into the regulatory relationship between cholesterol and the world of lipid transport.

A persuasive association of statistics about cardiovascular outcomes and levels of blood plasma lipids has created a sophisticated range of therapeutic targets for cholesterol lowering therapies [1].

Statin drugs are extensively used and are very effective in lowering serum low-density lipoprotein cholesterol [2]. They have been shown to reduce the incidence of cardiovascular events especially in secondary prevention, although there is reason to believe that most of their effects are mediated in spite of their cholesterol lowering action [3].

De-novo cholesterol, the target of statin therapy, is found in all membranes and lipid based bodies, where it is now known to be vital to their proper structure and operation. Ikonen's excellent review of 'cholesterol trafficking' [4] summarises the processes and mechanisms by which cholesterol contributes to vesicle formation, migrations and membrane functions throughout the cellular apparatus, and also illustrates the importance of cholesterol homeostasis. The function and adequacy of cholesterol in lipid membranes directly influences the production, secretion, delivery and utilisation of every lipoprotein [5].

By regulating the biosynthesis of cholesterol we potentially change the form and function of every membrane from the head to the toe. Statins created a potent medical opportunity along with potential for harm [6]. The past decade of research has exposed the nature of cholesterol-rich membrane rafts, raising fundamental clinical implications in neurology, immunology and all areas where lipoproteins are created, secreted and utilised. Our appreciation of cholesterol now extends far beyond the statistical link with cardio-vascular outcomes [7].

Cholesterol and insulin

Xia *et al*. inhibited a late step in the biosynthesis of de-novo cholesterol in murine and human pancreatic β cells [8] and published their findings in 2008. They had previously shown that insulin secretion was sensitive to the acute removal of membrane cholesterol. They now demonstrate that the depletion of membrane cholesterol impairs calcium voltage channels, insulin secretory granule creation, and mobilisation and membrane fusion.

This paper [8] clearly demonstrates that a direct causal link exists between membrane cholesterol depletion and the failure of insulin secretion. Their work is in close accord with data from some statin trials, which also connect cholesterol reduction with increased risk of type 2 diabetes; indeed, statin use has been shown to be associated with a rise of fasting plasma glucose in patients with and without diabetes [9]. The underlying mechanisms of the potential adverse effects of statins on carbohydrate homeostasis are complex [10] and might be related to the lipophilicity of the statin [11]. Indeed, retrospective analysis of the West of Scotland Coronary Prevention Study (WOSCOPS) revealed that 5 years of treatment with pravastatin reduced diabetes incidence by 30% [12]. The authors suggested that although lowering of trigliceride levels could have influenced diabetes incidence, other mechanisms such as anti-inflammatory action might have been involved; however, in the multivariate Cox model, baseline total cholesterol did not predict the development of diabetes [12]. Furthermore, pravastatin did not decrease diabetes incidence in the LIPID trial which included glucose-intolerant patients [13]. On the other hand, in the JUPITER trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin), which studied apparently healthy persons without hyperlipidemia but with elevated high-sensitivity C-reactive protein levels [14], the risk of diabetes was increased by a factor of 1.25 [95% confidence interval (CI), 1.05 to 1.51] among individuals receiving rosuvastatin 20 mg daily with respect to placebo. Strikingly, among persons assigned to rosuvastatin, the median low density lipoprotein (LDL) cholesterol level at 12 months was 55 mg per deciliter [interquartile range, 44 to 72 (1.1 to 1.9)].

It is intriguing that salutary lifestyle measures, which might exert their beneficial action through an anti-inflammatory mechanism without a strong cholesterol-lowering effect, beyond reducing cardiovascular events and total mortality, reduce also the risk of diabetes and other chronic degenerative diseases. This fact may represent a 'justification' not to use a drug in low-risk primary prevention populations: lowering cholesterol at the expense of increasing diabetes might be counter-productive over the long-term.

Cholesterol-rich membrane rafts

The role of cholesterol in cellular function became evident with the advent of the lipid raft hypothesis [15]. The original lipid raft hypothesis proposed the existence of assemblies of specific lipids, that compartimentalise the plasma membrane into functionally distinct areas [15, 16] involved in protein sorting events in polarized cells. It has now been clarified that lipid rafts are cholesterol- and sphingolipid-enriched membrane microdomains that function as platforms that concentrate and segregate proteins within the plane of the bilayer [17]; they are now thought to regulate membrane trafficking in both the exocytotic and endocytotic pathways, cell migration, and a variety of cell signalling cascades [18].

Lipid rafts consist of both protein and lipid components existing in continuity with non-raft regions of membrane. Lipid-lipid interactions seem to be of fundamental importance to the formation of lipid rafts, with cholesterol playing a special role as the 'glue' that holds these domains together [19].

The physical consequence of cholesterol depletion in membranes is dramatically illustrated by the experimental modelling work of de Meyer *et al*. [20]. They were able to demonstrate the manner in which cholesterol is uniquely able to influence the structure, thickness, permeability, deformation and other behaviours of membranes. A state of ordered stability is attained in cholesterol-rich lipid rafts when the level reaches 20-30% molecular cholesterol.

On the other hand, disorder, weakness and permeability might be created in cholesterol depleted membranes areas: cholesterol depletion inhibiting regulated exocytosis is a key discussion point in the review by Salaün *et al*. [21]. Molecule for molecule, cholesterol can make up nearly half of the cell membrane in lipid raft areas, cholesterol typically makes up 20% of total lipid molecules in the membrane [22]. Just for example, a relatively small depletion (< 10%) in synaptosomal membrane cholesterol has been shown to be enough to inhibit the release of a neurotransmitter [23].

Synaptogenesis and neural cholesterol

Nowhere is the impact of cholesterol depletion more keenly studied than in the neurologic arena.

The work of Pfrieger *et al*. described the functional role of cholesterol in memory through synaptogenesis [24]. Mauch *et al*. [25] reported evidence that cholesterol is vital to the formation and correct operation of neurons to such an extent that neurons require additional sources of cholesterol to be secreted by glial cells. A recent mini-review by Jang *et al*. describes the synaptic vesicle secretion in neurons and its dependence upon cholesterol-rich membrane areas of the synaptic membrane [26]. Furthermore, working on rat brain synaptosomes, Waseem [23] demonstrated that a mere 9.3% decrease in the cholesterol level of the synaptosomal plasma membrane could inhibit exocytosis. These data might be particularly worrisome for lovastatin and simvastatin which are known to cross the blood brain barrier [27].

In fact, the proposed use of statins as a therapeutic agent in Alzheimer's disease (AD) [28] counters Pfrieger's evidence [24]. Indeed, a reduction in cholesterol synthesis leads to depletion of cholesterol in the lipid rafts – i.e. the de-novo cholesterol is required in the neurons for synaptic function and also in the neuronal membrane fusion pores [29].

Cognitive problems are the second most frequent type of adverse events, after muscle complaints, to be reported with statin therapy [30] and this has speculatively been attributed to mitochondrial effects. The central nervous sytem (CNS) cholesterol is synthesised in situ and CNS neurons only produce enough cholesterol to survive. The substantial amounts needed for synaptogenesis have to be supplemented by the glia cells. Having previously shown that in rat retinal ganglion cells without glia cells fewer and less efficient synapses could form, Göritz *et al*. [31] indicate that limiting cholesterol availability from glia directly affects the ability of CNS neurons to create synapses. They note that synthesis, uptake and transport of cholesterol directly impacts the development and plasticity of the synaptic circuitry. We note their very strong implication that local de-novo cholesterol synthesis in situ is essential in the creation and maintenance of memory.

There should be further consideration of cholesterol depletion on synaptogenesis, behaviours and memory loss for patients undergoing long-term statin therapy. This is particularly important with lipophilic statins which easily cross the blood brain barrier [32].

The effects of statins on cognitive function and the therapeutic potential of statins in Alzheimer's disease are not clearly understood [28]. Two randomised trials of statins versus placebo in relatively younger healthier samples (lovastatin in one, simvastatin in other) showed significant worsening of cognitive indices relative to placebo [33, 34]. On the other hand, two trials in Alzheimer samples (with atorvastatin and simvastatin respectively) suggested possible trends to cognitive benefit, although these appeared to dissipate at 1 year [35, 36]. A recent Cochrane review concluded that there is good evidence from randomised trials that statins given in late life to individuals at risk of vascular disease have no effect in preventing Alzheimer´s disease or dementia [37]. However, case reports and case series from clinical practice in the real world reported cognitive loss on statins that resolved with discontinuation and recurred with rechallenge [30].

Evidence from observational data and prestatin hypolipidemic randomised trials showed higher hemorrhagic stroke risk with low cholesterol [30]. In fact, in the Stroke Prevention with Aggressive Reductions in Cholesterol Levels (SPARCL) trial as compared with placebo, the use of high-dose atorvastatin was associated with a 66% increase in the relative risk of hemorrhagic stroke among the patients receiving the statin drug [38]. In addition to treatment with atorvastatin, an exploratory analysis of the SPARCL trial found that having hemorrhagic stroke as an entry event, male sex, and advancing age at baseline accounted for the great majority of the increased risk of hemorrhagic strokes [39]. However, a sensitivity analysis excluding all patients with a hemorrhagic stroke as an entry event in the SPARCL trial found that statin treatment was still associated with an increased risk of hemorrhagic stroke [40]. Furthermore, in a subgroup of patients with a history of cerebrovascular disease enrolled in the Heart Protection Study [41] which did not include patients with hemorrhagic stroke, a similar increased risk of hemorrhagic stroke during follow-up was demonstrated [40].

Cholesterol in myelination and multiple sclerosis

The process in which axons are protected by the myelin secretions of the oligodendrocyte requires a specialised cholesterol-rich membrane [42]. Klopfleisch *et al.* [43] describe experimental *in vivo* evidence that new myelin (re-myelination) secretion by oligodendrocytes is impaired by statins.

Whilst they attribute much of this failure to signalling interference, they also prevented detrimental outcomes in vitro by re-incubating oligodendrocytes with cholesterol. How long are oligodendrocytes able to repair and maintain myelin in an environment where cholesterol is depleted?

It has been argued that statins can prevent demyelination [44] through a pleiotropic anti-

inflammatory effect and this has led to research on its use as a multiple sclerosis therapy.

This would appear to contradict Klopfleisch's findings [43], until you consider that initially there may be multiple conflicting effects over different time scales: Possibly the initial inhibiting of an auto-immune action associated with a de-myelination and subsequent inhibition of oligodendrocyte repairs by cholesterol depletion.

Research is needed to establish whether the apparent initial slowing of de-myelination in statin therapy would be followed by a catastrophic failure of the re-myelination work of oligodendrocyte exocytosis [45] as cholesterol synthesis fails. Furthermore, consideration should be given to the structural state of membranes involved in any auto-immune process where a complex interplay of essential membrane lipids, mediated by cholesterol, affects the immune response [46].

Neuro-muscular junctions and cholesterol

Symptoms associated with the malfunctioning of neuromuscular junction have frequently been reported by patients undergoing cholesterol lowering therapies [30]. A LDL receptor, called Lrp-4, is secreted by the neuro-muscular junction and it forms a complex with agrin which binds the muscle fibre receptor MuSK [47, 48]. The exocytoses of Lrp4 and agrin are active transport events, mediated through a cholesterol-rich lipid membrane. The secretion of the trans-membrane MuSK protein also requires a cholesterol-rich membrane raft.

There is extensive evidence to suggest that the depletion of cholesterol in both the synapse and post-synapse components of the neuro-muscular membranes areas would cause the failure of MuSK, Lrp4 and agrin exocytosis [49]. Such a failure would produce a myasthenic syndrome [50] with symptoms similar to those defining myasthenia gravis [51-55] and amyotrophic lateral sclerosis [56, 57].

Cholesterol and behaviours

The neurological effects of cholesterol depletion can produce a wide range of mental conditions reported to be associated with serum cholesterol depletion. Depression, violent behaviour, homicidal behaviour and suicide are all known associates of cholesterol depletion [58, 59].

In a recent study, cholesterol content was measured in cortical and subcortical tissue of brains from 41 male suicide completers and 21 male controls. Violent suicides were found to have lower gray matter cholesterol content overall compared with nonviolent suicides and controls [60].

Randomised trials with statins have not shown a definite association between cholesterol-lowering treatment and non-illness mortality from suicides, accidents, and violence [61, 62]. However, statin trials are specifically designed to test drug efficacy, often with run-in phases, and investigators usually conduct the studies in groups of patients who have few comorbidities and are not using many concomitant medications, and when side effects are measured, their seriousness and severity are not graded. Indeed, in clinical practice it has been suggested that severe anger and irritability may occour in some statin users [63].

Neural systems have significant vulnerability to cholesterol depletion. First is the reduction in the synaptic exocytosis and endocytosis of essential signalling lipoproteins; then comes the vulnerability due to the high dependency of myelination on de-novo cholesterol biosynthesis.

Membrane cholesterol and immunology

There are many immunologic functions that are dependent upon exocytosis, mediated by cholesterol-rich lipid rafts. There is an accumulation of exosome-sourced cholesterol caused by the infiltration of activated T lymphocytes into an atherosclerotic plaque as part of the immune response [7, 64].

In fact, statins affect multiple cell populations relevant to the immune response [65]. Although statins has been rarely associated with autoimmune disorders [30], the Trial of Atorvastatin in Rheumatoid Arthritis (TARA) study showed that atorvastatin (40 mg daily for 6 months) mediated modest but clinically apparent antiinflammatory effects in patients with rheumatoid arthritis [66]. However, the observed clinical efficacy was marginal in relation to both conventional disease-modifying antirheumatic drugs and novel biological compounds. Furthermore, statins seem to act in a disease-specific manner and are not effective in each immune disorder [65].

Peterson *et al*. [67] describe how the exocytosis of apolipoprotein B, very-low density lipoprotein (VLDL) and LDL secretions in skin protects against *Staphylococcus aureus* infection by interfering with the quorum sensing receptors which are needed to up-regulate the genes required for invasive infection. In this context we note a previous letter of Goldstein *et al.* [68] highlighting the possibility of a link between invasive methicillin resistant (MRSA) infection and statin therapy, when commenting on recent epidemiological trends.

Although no prospective randomised human trials testing the effects of statins in sepsis exist, it has been suggested that statins, blocking the inflammatory response associated with sepsis, might be of potential benefit [69]. However, mounting evidence suggests that the initial and intense systemic inflammatory response in patients, responsible for organ dysfunction and hypoperfusion

is accompanied by an anti-inflammatory process, acting in a negative-feedback manner. These inhibitory mechanisms could become harmful since nearly all immune functions are compromised, and therefore they may account for the majority of deaths after sepsis [70]. Moreover, it has been shown that hypocholesterolemia in critical illness and multisystem organ failure correlates with decreased patient survival rates [71]; lipoproteins have been found to bind with and neutralize bacterial endotoxins [72]. Indeed, favorable results of lipid-infusion therapy have been noted in some animal studies [73, 74].

The immunomodulatory action of statins might also be seen as a double-edged sword because it may also hinder the host anti-tumor immune response, therefore increasing cancer risk [75].

A recent systematic review [76] found that statins do not have short-term effects on cancer risk. However, the strength of evidence was weak, and these data was mostly derived from randomised trials of short duration and related to highly select people; thus the extrapolation to patients seen in clinical practice should not be considered straightforward. In particular, the elderly, who have depressed immune functions and are more likely than younger subjects to harbor microscopic foci of cancer cells, might be particularly subject to adverse outcomes from the immunosuppressive effects of statin therapy [77].

The results of the Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) trial have been recently published [78]: during a follow-up of 52.2 months, simvastatin and ezetimibe, as compared to placebo, did not reduce the composite outcome of combined aortic-valve events and ischemic events in patients with aortic stenosis. However, of more concern, an excess of incident cancers was observed in the simvastatin-ezetimibe group, with 105 in that group as compared with 70 in the placebo group (p = 0.01). Also, deaths from cancer were more frequent in the active-treatment group (39 deaths, vs. 23 in the placebo group), achieving a borderline statistical significance (p = 0.05). Of note, the average age in the SEAS trial was 68 years.

In this setting, beyond the immunomodulatory effect of simvastatin which might promote growth increase of occult cancers, it cannot be dismissed the action of ezetimibe which inhibits the absorption of phytosterols and other phytonutrients that are linked to protection against cancer [79].

Cholesterol and bone fractures

Studies associating statin therapies with reductions in bone loss conflict with those reporting an association with bone fractures [80]. If statin therapies are down regulating lipid trafficking, bone remodelling might be slowed. Osteoclasts, when depleted of membrane cholesterol, will be restricted in their ability to absorb old bone matrix, thereby appearing to protect against bone loss [81]. Likewise, osteoblasts, when depleted of membrane cholesterol, will be restricted in their ability to secrete new mineral matrix into fractures. Interestingly, higher total serum cholesterol levels have been shown to protect against fractures in post-menopausal women at risk of osteoporosis [82].

Conclusions

We are now realizing that the intricate connection between endocytosis and exocytosis, cholesterol-rich lipid membranes and the trafficking of lipoproteins within and between cells is the key to understanding the benefits and detriments of cholesterol lowering therapies. Current guidelines encourage aggressive and long-term cholesterol lowering with statins, in order to decrease cardiovascular disease events [1]. The main benefits of this therapy are thought to be due to plaque stabilization in the arterial wall [83]. However, cholesterol lowering alters cell membranes from head to toe, the implication of which may be good, bad or neither. Most importantly, more research is needed in this field, as wider segments of the population are exposed to aggressive cholesterol lowering. This research should answer the question: Is it possible, with aggressive cholesterol lowering, to achieve long-term plaque stability and simultaneously maintain cellular membrane integrity and function?

It has recently been shown that high LDL cholesterol is not a major cause of death at the population level [84]. Changing our current practice pattern could take many years, but we may one day prescribe cholesterol-raising medications to certain patients [85].

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