Might the beneficial effects of statin drugs be related to their action on iron metabolism?

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Summary

Although the cholesterol-heart hypothesis is often regarded as a dogmatic belief, controversy continues to surround the aetiology and pathogenesis of atherosclerosis. In fact, lowering cholesterol with statin drugs has been shown to reduce cardiovascular risk. However, statins have pleiotropic effects independent of their capacity to lower cholesterol. We highlight that statin drugs exert an important action on iron metabolism, which in turn may prevent progression and destabilization of atherosclerotic plaque. If it is found that the effect of statins on iron metabolism is a mechanism of their beneficial action, this consequence of statin use can be clinically replicated by other methods, such as controlled reduction of body iron stores. This might allow the use of lower doses or even obviate the use of statins in primary cardiovascular prevention, and therefore avoid the side effects and expense of these drugs.

Introduction

It is widely believed that the connection between cholesterol elevation and atherosclerotic plaques (with related cardiovascular events) is clear and well established, with the consequent assumption that ‘atherosclerosis is a cholesterol problem’. However, healthy premenopausal women are largely protected from ischaemic heart disease, but, remarkably, so are women with heterozygous familial hypercholesterolemia. Cardiovascular protection despite a grossly unfavourable lipid phenotype suggests that the protective factor is not only powerful but also does not operate through a lipid-related mechanism. In fact, this pattern argues against the earlier view that oestrogen is the protective factor because of its effects on the lipid profile. Randomized trials of hormone replacement therapy in women without familial hypercholesterolemia show that oestrogen or the combination of oestrogen and a progestin significantly improves lipid profiles, but fails to protect against atherosclerosis or cardiovascular events. Furthermore, heart protection by premenopausal levels of female hormones is not consistent with epidemiological findings that premenopausal hysterectomy essentially cancels the protection, even in cases with preservation of functioning ovaries.

On the contrary, these data suggest that an intact uterus has an important role in the protection of premenopausal women, and likely this is related to the beneficial effect of iron depletion in menstruating women (i.e. the iron hypothesis suggested by Sullivan more than 30 years ago).

The ‘iron hypothesis’

In late adolescence, men begin a steady accumulation of storage iron with age, but women fail to acquire significant iron stores because of their...
continual losses of iron in menstrual blood, pregnancies and deliveries. An escalation of risk follows initial acquisition of significant stored iron after cessation of menses due to natural menopause, or to surgical removal of the uterus and/or the ovaries.

A protective effect of iron depletion that may have multiple beneficial consequences is decreased availability of redox-active iron in vivo. The amount of free iron available at sites of oxidative or inflammatory injury appears to be a function of the stored iron level. Removal of stored iron from the body by phlebotomy, systemic iron chelation treatment or dietary iron restriction has been shown to decrease the amount of iron deposition within atherosclerotic lesions in animal studies. In particular, in experimental mice the iron chelator desferrioxamine inhibited inflammation and atherosclerotic lesion development, thus providing the proof-of-concept that iron plays an important role in the pathogenesis of atherosclerosis-mediated clinical events. In fact, epidemiological observations also suggest a role of iron depletion in cardiovascular protection: (i) lower stored iron level mediated by cyanosis-induced hypoxia may explain why cyanotic patients with congenital heart disease might be protected from atherosclerosis; (ii) the protection against ischaemic cardiovascular disease in individuals with impaired haemostasis might be related to the decrease of stored tissue iron caused by recurrent bleeding.

A persistent criticism of the iron hypothesis has been that atherosclerosis is not a prominent feature of homozygous hemochromatosis. The essence of this criticism is that iron cannot be a significant factor in atherogenesis in those unaffected by inherited iron overload unless an increase in atherosclerosis is observed in hemochromatosis. However, the emerging details of the physiology of hepcidin, the key hormone in iron balance and iron recycling, suggest a resolution of the apparent paradox of an important role for iron in atherogenesis in the absence of increased plaque burden in homozygous hemochromatosis. Hepcidin acts to block both iron absorption in the gut and iron release from macrophages through a common mechanism. Ferroportin is the sole known iron exporter in enterocytes and macrophages. Hepcidin binds ferroportin on cell membranes causing its internalization and degradation. Hepcidin levels are upregulated by iron intake and inflammation and markedly downregulated by iron deficiency anaemia. Very low hepcidin levels are also observed in most cases of hereditary hemochromatosis. Because of the low hepcidin levels in the two conditions, iron deficiency anaemia and hereditary hemochromatosis are both characterized by macrophages with little or no iron. The failure of vascular wall macrophages to retain iron in cases of inherited iron overload may prevent progression and destabilization of atherosclerotic plaque.

Therefore, the physiology of hepcidin suggests a novel and specific mechanism by which iron depletion could promote the stability of atherosclerotic plaques. This interaction involves effects of hepcidin on excessive iron deposition in the macrophages within atherosclerotic plaque with subsequent increased lipid peroxidation and progression to foam cells. The low hepcidin levels found in inherited hemochromatosis may explain the paradoxical effects of iron overload and decreased atherogenesis in patients with hemochromatosis.

The nonuniform effect of cholesterol-lowering drugs on cardiovascular risk

On the other hand, no major randomized clinical trial has tested the benefits of treating patients according to their cholesterol targets. Fixed doses of cholesterol-lowering drugs have been tested in selected populations. Among these trials, only statins have been shown to reduce cardiovascular risk. Other cholesterol-lowering drugs have failed to reduce risk, such as clofibrate and torcetrapib, or remain to be tested, such as ezetimibe. Therefore, because evidence from randomized trials has shown that not all drugs that lower cholesterol levels reduce patients’ cardiovascular risk, it might be hypothesized that some of the beneficial effects of statin drugs are not related to their cholesterol-lowering action. In this setting, we suggest that statins may reduce cardiovascular risk through their effect on iron metabolism. This hypothesis is also consistent with the natural protection seen in menstruating women with heterozygous familial hypercholesterolemia, as previously mentioned.

Action of statins on iron metabolism

Heme-oxygenase (HO) is the rate-limiting enzyme in the catabolism of heme. Heme catabolism represents a key function in mobilizing macrophage iron derived from ingested erythrocytes. Importantly, the storage and processing of iron from erythropagocytosis by macrophages within plaque appear to play a key role in plaque progression. Accordingly, it has been demonstrated that erythrocytes induce plaque vulnerability in a dose-dependent manner in a rabbit model of intraplaque hemorrhage.
HO catalyses heme degradation to iron, carbon monoxide and biliverdin\textsuperscript{19} (Figure 1). HO-1 is the inducible isoform of HO, which plays an important role as an anti-oxidant and anti-inflammatory.\textsuperscript{20} Whereas the constitutive HO isoenzyme, HO-2, might afford some level of protection against free heme, its expression is not inducible in response to oxidative stress, which probably makes HO-2 less likely to play a central role in affording cytoprotection against free heme.\textsuperscript{21} On the other hand, it has been found that HO-1 contributes to the protection from vascular inflammation in atherosclerosis.\textsuperscript{22}

Alterations in the activity of HO-1 influence the rate of clearance of hemoglobin-derived iron from macrophages. In the HO-1 deficient mouse (HO-1\textsuperscript{-/-}), conspicuous iron loading is found in Kupffer cells, hepatocytes, hepatic vascular tissue and renal cortical tubules.\textsuperscript{23} Another study involving HO-1\textsuperscript{-/-} mice showed increased levels of reactive oxygen species production in macrophages and increased atherosclerotic plaque.\textsuperscript{24} These pathological findings could have been related to a relatively decreased intracellular levels of biliverdin or bilirubin and increased intracellular levels of iron stores. Not surprisingly, pharmacologic inhibition of HO-1 with Sn protoporphyrin and Sn mesoporphyrin is associated with a significant suppression of bilirubin production, lower serum and biliary bilirubin levels and increase biliary heme output\textsuperscript{25}: in normal subjects, levels of serum ferritin, but not those of other acute phase reactants, increased substantially but transiently after administration of these HO inhibitors.

Statins, by blocking the conversion of 3-hydroxy-3-methylglutaryl coenzyme A into mevalonate, decrease the level of isoprenoids, thus reducing prenylation of proteins.\textsuperscript{26} The prenylation of proteins, such as the small G-proteins Ras, Rho and Rac is essential for their translocation to the cell membrane and therefore their activity. Furthermore, statins are able to modulate cellular function directly at transcriptional level. Through these actions, statins modulate the activity of many transcription factors essential for gene expression. In this setting, statin drugs have been reported to induce HO-1 in murine macrophages\textsuperscript{27} and in human endothelial cells.\textsuperscript{28} Furthermore, in murine embryonic fibroblast cells simvastatin and fluvastatin have been shown to activate HO-1 gene transcription, and increase HO-1 promoter activity.\textsuperscript{29} Of note, fluvastatin treatment has also been found to lower serum prohepcidin levels in patients with end-stage renal disease.\textsuperscript{30}
whereas simvastatin significantly suppressed the mRNA expression of hepcidin in HepG2 cells.\textsuperscript{31} In addition, statins have been reported to lower concentrations of interleukin-6,\textsuperscript{32} a potent inducer of hepcidin, which has been recently confirmed to represent a positive regulator of atherosclerotic plaque destabilization via regulating iron homeostasis in macrophages.\textsuperscript{33}

It is noteworthy that interaction of serum ferritin with statin use has been found in a substudy of the iron (Fe) and atherosclerosis study.\textsuperscript{34} At baseline, 53 participants on statins had slightly lower mean entry-level ferritin values (114.06 ng/ml) vs. the 47 off statins (127.62 ng/ml). Longitudinal analysis of follow-up data, after adjusting for the phlebotomy treatment effect, showed that statin use was associated with significantly chronic lower ferritin levels (–29.78 ng/ml; $P = 0.02$).

**Conclusion**

Questioning conventional wisdom may appear difficult or uncomfortable. However, although the cholesterol-heart hypothesis is often regarded as a dogmatic belief, controversy continues to surround the aetiology and pathogenesis of atherosclerosis.\textsuperscript{35} In fact, it has been suggested that hypercholesterolaemia does not represent a *sine qua non* for the development of atherosclerosis, and that the beneficial effects of statins do not resolve the cholesterol controversy.\textsuperscript{36} Statins have pleiotropic effects independent of their capacity to lower cholesterol. The failure of vascular wall macrophages to retain iron related to statin-induced intralesional HO-1 activation, accompanied by reduced hepcidin expression may prevent progression and destabilization of atherosclerotic plaque.

If it is found that the effect of statin drugs on iron metabolism is a predominant mechanism of their beneficial action, this consequence of statin use can be clinically and safely replicated by other methods, such as a controlled and continued reduction of body iron stores by phlebotomy,\textsuperscript{37} to reach ferritin levels of 17–25 ng/ml as suggested by Sullivan’s original observations of levels in menstruating women.\textsuperscript{6} This might allow the use of lower doses or even obviate the use of statins in primary cardiovascular prevention, and therefore avoid the side effects—easily dismissed and not trivial in real life,\textsuperscript{38,39} and expense of these drugs.

**Conflict of interest:** None declared.


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