Biological consequences of statins in *Candida* species and possible implications for human health

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Abstract

The statins, simvastatin and atorvastatin are the most widely prescribed drugs. Statins lower cholesterol levels through their action on HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, an essential enzyme for the biosynthesis of cholesterol. Fungal HMG-CoA reductases are also inhibited by statins, resulting in reduced levels of ergosterol (the fungal equivalent of cholesterol) and concomitant growth inhibition. This effect occurs in a range of fungal species and possibly affects fungal colonization of people on statin therapy. Furthermore, it may suggest that statins could have a role in new antifungal therapies. Possibly associated with the reduction in ergosterol levels, statins also inhibit respiratory growth. In the yeast, *Candida glabrata*, passage with statins dramatically increased the frequencies of petite mutants that were devoid of mitochondrial DNA, suggesting that statins caused a defect in the maintenance of mitochondrial DNA. These observations in *C. glabrata* may provide further insights into side effects of statins in humans undergoing treatment for hypercholesterolaemia. In addition, *C. glabrata* may be highly useful for the preliminary screening of agents to reduce statin side effects.

Introduction

Statins, the most prescribed of all current drugs, are used to reduce serum cholesterol levels in humans. They inhibit the enzyme HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, which catalyses the conversion of HMG-CoA into mevalonate (see Figure 1). In addition to competing with HMG-CoA for the enzyme active site [1], statins change the conformation of the enzyme on binding, preventing HMG-CoA reductase from attaining a functional structure [2].

Along with the lowering of cholesterol, statins have other beneficial effects including improvement of endothelial function and reduction of inflammation in atherosclerotic plaques [3–5]. Statins are also reported to reduce the risks of coronary heart disease [6] and stroke [7] and help in cancer therapy [8–11]. Both *in vitro* and *in vivo* studies have also demonstrated that statins inhibit tumour growth and induce apoptosis in a variety of tumour cells [12]. In Alzheimer’s disease, the statins reduce intracellular and extracellular levels of the amyloidogenic peptides Aβ42 (amyloid β-peptide 42) and Aβ40 [13], while another study determined that reduced levels of Aβ in the brains of patients suffering from Alzheimer’s disease were due to lower cholesterol levels [14]. Overall, many studies conclude that statin therapy lowers the risk of Alzheimer’s disease [15–17].

Statins inhibit the synthesis of mevalonate, which is the precursor for many other compounds in addition to cholesterol (see Figure 1); therefore statins may be expected to inhibit the production of dolichols, CoQ (coenzyme Q; ubiquinone), haem A and prenylated proteins, resulting in pleiotropic effects. Indeed, statins can cause severe side effects such as hepatotoxicity [18,19] and myopathy.

Statin effect on growth, ergosterol synthesis and fungal colonization

Yeast and particularly *Saccharomyces cerevisiae* is a validated model for the study of cellular processes that are conserved in all eukaryotic cells [20,21]. Yeasts possess the biosynthetic pathway to produce mevalonate, dolichols, CoQ, haem A and prenylated proteins (see Figure 1). The only difference from humans is that in yeast the end-product of the ‘main pathway’ is ergosterol, a close structural and functional relative of cholesterol [20]. It is also expected that inhibition of any of the pathways is likely to be detrimental to the cell.

Some of the older statins, compactin and lovastatin have been shown to inhibit ergosterol synthesis [22]. It has been shown that treatment with simvastatin in *Candida glabrata* produced a significant lowering of ergosterol and a concomitant growth inhibition [23]. However, growth could be almost fully restored by the addition of ergosterol or cholesterol [23]. Statin-induced growth inhibition can be best observed in
minimal media (compared with rich media) where the absence of ergosterol enhances the inhibition by the statin (also see Figure 2A). Results with atorvastatin are the same, as are the effects on Candida albicans (results not shown). Simvastatin and atorvastatin also inhibit the growth of a variety of pathogenic Candida species and Aspergillus fumigatus [23]. Interestingly, however, not all fungi are affected to the same extent. C. albicans and C. glabrata were the most inhibited, while Candida krusei was only moderately inhibited. These results led to our suggestion that fungal colonization may be affected in statin-treated people [23]. Indeed, simvastatin levels in sera can be several micromolars [24], a level that could moderately inhibit the growth of the more sensitive Candida species such as C. albicans.

There is circumstantial and emerging evidence that statins do affect fungal colonization. In a recent study, Kopack and Forrest [25] showed that statins affect outcomes in candidaemia. For patients with candidaemia, those who received statins showed a survival rate three times higher than those not on statins. In addition, those on statins were less frequently colonized by C. albicans. These results are comparable with our results [23] which showed greater sensitivity of C. albicans to statins. There is a need for further epidemiological studies to determine the effect of statins on Candida and indeed other fungal infections. If indeed statins do inhibit colonization by some yeasts, it would be worth pursuing further exploration of a range of statins, possibly at high short-term doses, to determine whether they may have a more general role in the treatment of fungal infections.

Statin effects on yeast mitochondrial functions

The growth of Candida species in various growth media with simvastatin and atorvastatin has also shown that mitochondrial function is affected by statins [23]. On rich media with glucose as a carbon source [YEPD (yeast extract/peptone/dextrose)], statins caused minor growth inhibition (Figure 2A). However, when ethanol replaced glucose as the carbon source [YEPE (yeast extract/peptone/ethanol)], the statins severely inhibited growth. Ethanol is a non-fermentative substrate requiring respiratory function for its utilization and hence it can be concluded that statins inhibit respiratory function. This inhibition applies, in varying degrees, to all Candida species that have been tested.

Of particular interest is our further investigation of the effects of statins on C. glabrata species. This yeast is petite-positive, so it can still grow even with a respiratory defect resulting from an mtDNA (mitochondrial DNA) deletion mutation. Mutations in mtDNA are non-reverting and in C. glabrata such mutations occur spontaneously at a very low frequency of one in 10⁵ cells [26]. We found that simvastatin and atorvastatin induced petite formation at a very high frequency; almost 100% of the culture became petite after as little as 3 days culture with simvastatin [23]. Furthermore, unlike regular spontaneous petites that undergo specific deletions in their mitochondrial genome and amplify the remaining DNA (so-called ρ− petites), statin-induced petites are totally devoid of mtDNA and are designated ρ0. DAPI (4′,6-diamidino-2-phenylindole) staining of cellular DNA of
petites showed that only nuclear DNA was present in such cells [see Figures 2B and 2C] [27].

How might statins affect mtDNA maintenance? Loss of mtDNA from cells via unequal segregation could lead to loss of mtDNA in daughter cells, or defective mtDNA replication could cause the loss of mtDNA. Membrane-perturbing agents such as ethanol can result in ergosterol reduction and associated petite induction in S. cerevisiae [27]. It has previously been shown that the replication of mtDNA is membrane-associated [28]. Thus there is already a potential link between ergosterol levels and mtDNA loss. Another explanation could be that decreased production of farnesyl P-P could lead to reduced levels of CoQ, which plays an important role in mitochondrial function and as an antioxidant [29,30]. Reduced protection from oxidative damage could lead to mtDNA damage; mtDNA is more sensitive than nuclear DNA to oxidative damage [29].

Schneiter [31] used a genetic approach to examine the relationship between ergosterol biosynthesis and mitochondrial biogenesis by screening yeast genes required in anaerobic growth [31]. Under anaerobic conditions, yeast are auxotrophic for sterols. His screening showed that 17 genes involved in sterol uptake and/or transport are also involved in mitochondrial functions [32]. Mutants deficient in ergosterol biosynthesis showed swollen and clumped mitochondria [27]. All these point to a significant interconnection between the mevalonate pathway and mtDNA maintenance in the yeast cell. Statins are also proposed to affect the plasma membrane in yeast that stores the vast majority of ergosterol in the cell [33]. Statins, in inhibiting mevalonate synthesis, might trigger a number of events that may further impact other cell functions and metabolic pathways.

Comparison of statin effects on human mitochondrial functions

Statins are employed to achieve cholesterol reduction and, in general, they do this very effectively and safely. It would appear that they are a medicine that prevents the development of life-threatening conditions. A consequence of the reductions in plasma cholesterol levels is the amelioration of vascular atherosclerosis [18,33]. They also increase the uptake and degradation of LDL (low-density lipoprotein) and prevent LDL oxidation [18,33]. Statins exhibit many anti-atherosclerotic effects, such as inhibition of macrophage growth, inhibition of cholesterol accumulation in macrophages, prevention of superoxide generation and increased activity and expression of the eNOS (endothelial nitric oxide synthase) gene in human endothelial cells [18]. Mevalonate plays a key role in cell proliferation and differentiation, and statins may be useful for preventing tumour formation by selective inhibition of mevalonate production [18].

However, because statins act on a complex pathway, they may also be expected to inhibit the production of dolichols, CoQ, haem A and prenylated proteins (see Figure 1). Thus there might be pleiotropic effects caused by statins on human health either due to cholesterol reduction or decrease in any of the above-mentioned enzymes. For example, the antiproliferative properties of statins have been suggested to result from reduced prenylation of Ras [34]. Similarly, reduced prenylation of BACE (β-amyloid-cleaving enzyme) has been suggested to be the mechanism by which statins might slow the progression of Alzheimer’s disease which appears to be related to Aβ levels [35]; prenylated BACE is the protease that releases Aβ from its precursor protein. In reality, the mechanism of the positive role of statins in these major diseases is still to be precisely determined.

There are also numerous reports of undesirable side effects of statins. Clearly, tight inhibition of HMG-CoA reductase is undesirable and the strongest HMG-CoA reductase inhibitor to be commercialized, cerivastatin, was withdrawn in 2001 following the death of 52 people [36]. This underscores the danger of reducing cholesterol synthesis to the point where cell damage results, and justifies the biennial testing of markers of hepatotoxicity and rhabdomyolysis for statin users.

The most common adverse effect to be reported is myopathy. Typical statin-induced myopathy is characterized by muscle pain, tenderness and weakness and is normally associated with increased serum creatine kinase levels, the biochemical marker of cell damage [37]. However, many statin users exhibit normal creatine kinase levels while still reporting the above symptoms [38]. Evidence of the large amount of subclinical pain and discomfort can be observed in a Google search of ‘statin+side effects’, which yields over 2 million hits; many of the hits relate to a downturn in various kinds of fitness.

It is tempting to speculate that inhibition of mitochondrial functions may account for the main statin side effects, most of which are the adverse effects on muscle cells. One theory involves the reduced synthesis of CoQ [37]. CoQ is involved in energy production via the mitochondrial respiratory pathway. Biopsy findings have demonstrated that muscle cells from statin users show mitochondrial respiratory dysfunction, indicated by increased lipid stores, cytochrome oxidase-negative myofibres and ragged red fibres [37]. The authors of this study [37] suggested that reduction in cholesterol in skeletal-muscle cells made the cell membrane unstable. Further, a reduction in prenylated proteins, including GTP-binding proteins that are important regulatory proteins in cell health and apoptosis, may lead to growth inhibition.

C. glabrata as a model for statin-induced mitochondrial side effects

The broad and often unexpected effects of statins are a testament to the need to further investigate statins. Such studies may provide rational approaches for novel uses of statins, as well as guide strategies to alleviate statin side effects.

A summary of statin effects on C. glabrata and a comparison with (possibly) related effects in mammalian cells is shown in Table 1. Overall, there are many similarities. Where there do appear to be differences, we consider the
Statins effects in yeast and mammalian cells

**Table 1** Statin effects in *C. glabrata* compared with mammalian cell lines

<table>
<thead>
<tr>
<th><em>C. glabrata</em></th>
<th>Mammalian cells</th>
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<tbody>
<tr>
<td>Ergosterol reduction [23,27]</td>
<td>Cholesterol reduction [18,40]</td>
</tr>
<tr>
<td>Loss of respiratory function; petite induction; mtDNA loss [23,27,39]</td>
<td>Loss of membrane potential; swelling of the mitochondria; inhibition of electron transport chain and mitochondrial oxidation [19,37]</td>
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<tr>
<td>Loss of respiratory growth; sterol auxotrophy [39]; death (K. Wikhe and I. Macreadie, unpublished work)</td>
<td>Growth inhibition due to requirement for mevalonate [41]; apoptotic cell death [19,34]</td>
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Differences can mostly be reconciled. For example, the cholesterol–ergosterol difference is precisely related. Statins... mitochondrial function in mammalian cells lines to our knowledge. Despite its difference, *C. glabrata*, and its mtDNA loss, could serve as one of the best models for examining statin side effects. Screens could be designed to look for compounds that lower the frequency of statin-induced petite formation.

**References**

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