Growth inhibition of *Candida* species and *Aspergillus fumigatus* by statins

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**Abstract**

Statins are a class of drugs widely used for lowering high cholesterol levels through their action on 3-hydroxy-3-methylglutaryl-CoA reductase, a key enzyme in the synthesis of cholesterol. We studied the effects of two major statins, simvastatin and atorvastatin, on five *Candida* species and *Aspergillus fumigatus*. The statins strongly inhibited the growth of all species, except *Candida krusei*. Supplementation of *Candida albicans* and *A. fumigatus* with ergosterol or cholesterol in aerobic culture led to substantial recovery from the inhibition by statins, suggesting specificity of statins for the mevalonate synthesis pathway. Our findings suggest that the statins could have utility as antifungal agents and that fungal colonization could be affected in those on statin therapy.

**Introduction**

Statins are the main therapeutic agents used to decrease high serum cholesterol levels and are among the most widely prescribed drugs currently on the market. They competitively inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase by binding to the active site of HMG-CoA reductase (Qiu et al., 1991), an enzyme that catalyses the conversion of HMG-CoA to mevalonate and subsequently to farnesyl diphosphate. Farnesyl diphosphate is the precursor for the production of cholesterol in humans or ergosterol in plants and eukaryotic microorganisms. If the statin-binding site of fungal HMG-CoA reductase is similar to that of human HMG-CoA reductase, it might be expected that statins or their derivatives could also be used to inhibit the growth of fungal pathogens through inhibition of ergosterol synthesis. This study examines the effects of the commonly prescribed statins, simvastatin and atorvastatin, on six species of pathogenic fungi.

**Materials and methods**

**Chemicals**

Simvastatin and atorvastatin were purchased from 7Chemicals (India). Tween 80, cholesterol and ergosterol were purchased from Sigma-Aldrich. To activate simvastatin, provided in the form of a lactone prodrug, it was hydrolysed in ethanolic NaOH [15% (v/v) ethanol and 0.25% (w/v) NaOH] at 60 °C for 1 h (Lorenz & Parks, 1990). Stock solutions of the hydrolysed simvastatin at a concentration of 20 mg mL⁻¹ were stored at −20 °C.

**Yeast strain and media**

The 13 yeast strains used in these studies were as follows: *Candida albicans* JRW#5, WM1172, ATCC90028 and CBS562; *Candida glabrata* ATCC90300, and CBS138; *Candida tropicalis* ATCC750, WM213 and WM30; *Candida krusei* ATCC6258, and WM03,204; *Candida parapsilosis* ATCC22019; and *Aspergillus fumigatus* strain 03.209-3938. Strains were grown in YEPD (1% yeast extract, 2% peptone, 2% dextrose), YEPE (1% yeast extract, 2% peptone, 2% ethanol) or in YNB (0.67% Difco yeast nitrogen base without amino acids, 2% glucose) media. For supplementation of media, Tween 80 (1:1 in ethanol) was added to a final concentration of 0.5%. Cholesterol and ergosterol were dissolved in the Tween 80/ethanol mixture and added to a final concentration of 12, 36 or 108 μg mL⁻¹.

**Yeast growth**

*Candida* and *Aspergillus* species in log-phase growth in YEPD media were suspended in water and then transferred
to solidified media for incubation at 30 °C for 2 days. Solidified media containing statins were freshly prepared before use, although statins remained active in solidified media stored at 4 °C for up to 10 days.

Results

Growth inhibition of Candida and Aspergillus species

The effect of simvastatin and atorvastatin on the growth of Candida species was measured on three types of solidified media: YEPD, YEPE and YNB media. Statin concentrations were between 0 and 1000 μM. The results of growth in the different media with different concentrations of statins are shown in Table 1 (and Fig. 1).

On rich media (YEPD), C. parapsilosis and all four C. albicans isolates were strongly inhibited by concentrations of 100 μM simvastatin or atorvastatin. One C. glabrata isolate (ATCC90300) exhibited low sensitivity to 300 μM atorvastatin and simvastatin, while the other (CBS138) similarly showed low sensitivity to 300 μM atorvastatin but was sensitive to 100 μM simvastatin. Inhibition within species appeared to be uniform as the four isolates of C. albicans, three of C. tropicalis and two of C. glabrata exhibited similar sensitivity profiles. Of the two isolates of C. krusei tested, minimal inhibition was observed; however, some sensitivity was apparent in one of these (ATCC6258) when exposed to 300 μM simvastatin.

Overall, when using YEPE, the sensitivity profiles obtained at 100 μM simvastatin and atorvastatin were similar to those observed when using rich media, although inhibition was found to be slightly stronger on YEPE. At concentrations of 1000 μM simvastatin, all of the yeast isolates were to be strongly inhibited, whereas atorvastatin, at this same concentration, showed less effective inhibition. Of two isolates of C. krusei tested, both showed complete inhibition at 1000 μM simvastatin but with no sensitivity to 1000 μM atorvastatin.

When minimal medium (YNB) was used, increased levels of inhibition were observed so in Table 1 we show the results with lower concentrations of statins. All Candida isolates tested on this medium showed significant inhibition at 100 μM statin levels, with the exception of the C. krusei isolates, which appeared resistant to 100 μM atorvastatin. In addition, many strains exhibited inhibition by concentrations as low as 3 μM simvastatin and 10 μM atorvastatin. The C. parapsilosis isolate was incapable of growth on minimal media.

The effects of statins on the growth of A. fumigatus, a filamentous fungal pathogen from a different genus, were also investigated on solidified minimal media. In the absence of statins, A. fumigatus exhibited robust growth with production of conidia after 4 days at 30 °C (Fig. 2). In the presence of statins, there was growth inhibition. Atorvastatin at 3 μM caused substantial growth inhibition (data not shown), with no growth being observed at 10 μM (Fig. 2), while simvastatin caused substantial growth inhibition at 0.1 μM (data not shown) and no visible growth at 1 μM (Fig. 2).

Rescue of A. fumigatus and C. albicans growth inhibition with sterols

As the action of the statins appears to be mediated through the sterol synthesis pathway, the action of the statins on growth inhibition was examined by testing rescue of growth inhibition with sterols. This was performed qualitatively for

### Table 1. Growth of Candida species in the presence of simvastatin and atorvastatin

| Candida species | Strain       | YEPD (μM) | | | | YNB (μM) | | | | YEPE (μM) | | |
|-----------------|--------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|                 |              | AVS      | SVS      | AVS      | SVS      | AVS      | SVS      | AVS      | SVS      | AVS      | SVS      |
| C. albicans     | JRW#5        | ++       | –        | –        | –        | –        | –        | ++       | –        | –        | –        |
| C. albicans     | WM1172       | +++      | –        | –        | –        | –        | –        | +        | –        | –        | –        |
| C. albicans     | ATCC90028    | +++      | –        | –        | –        | –        | –        | ++       | –        | –        | –        |
| C. albicans     | CBS562       | +++      | –        | –        | –        | –        | –        | +        | –        | –        | –        |
| C. glabrata     | ATCC90300    | +++      | ++       | ++       | ++       | ++       | ++       | +++      | ++       | –        | +++      |
| C. glabrata     | CBS138       | +++      | ++       | ++       | ++       | ++       | ++       | +        | ++       | +        | ++       |
| C. tropicalis   | ATCC750      | +++      | +        | ++       | ++       | ++       | ++       | +        | ++       | +        | +        |
| C. tropicalis   | WM213        | +++      | +        | +        | +        | +        | +        | +        | +        | +        | –        |
| C. tropicalis   | WM30         | +++      | +        | +        | +        | +        | +        | +        | +        | +        | –        |
| C. krusei       | ATCC6258     | +++      | +++      | +++      | +++      | +++      | +++      | +++      | ++       | +        | +        |
| C. krusei       | WM03204      | +++      | +++      | +++      | +++      | +++      | +++      | +++      | +++      | ++       | +        |
| C. parapsilosis | ATCC22019    | +        | –        | –        | –        | NA       | NA       | NA       | NA       | +        | –        |

Strains were grown with atorvastatin (AVS) or simvastatin (SVS) at the levels shown. Growth (strong, +++; moderate, ++; low, +; none, –; NA, not applicable) is shown after 2 days on YEPD, YNB or YEPE media.
After treatment with sterols for 4 days at 30 °C, *A. fumigatus* showed recovery from statin-induced growth inhibition (Fig. 2), although growth was slower compared with that of untreated *A. fumigatus*. Both ergosterol and cholesterol appeared equally effective in rescuing *A. fumigatus* growth following treatment of simvastatin and atorvastatin.

To test for sterol rescue of *C. albicans*, growth yields were measured after 18 and 42 h. Using concentrations of 3 μM simvastatin or 10 μM atorvastatin, growth yields were reduced to levels less than 2% of those obtained without addition of statins (Fig. 1a). When the media were supplemented with the ergosterol or cholesterol, growth levels recovered to ~50% of the levels where no statin was present after 18 h (Fig. 1a). Increasing the levels of either sterol to 108 μg mL⁻¹ sterol did not lead to complete growth recovery: the greatest growth obtained was ~70% of that with no statin (data not shown). However, we found that after extended culture, growth yields were much higher. Cells treated with 10 μM atorvastatin plus cholesterol or ergosterol achieved yields equal to the levels of the untreated culture, while cells treated with 3 μM simvastatin plus cholesterol or ergosterol reached yields of ~90% of the untreated culture (Fig. 1b). This would appear to indicate that rates of uptake of cholesterol or ergosterol are too slow to permit uninhibited growth, but with slow growth the sterol needs can be met by uptake. The combination of Tween 80 with a sterol was essential: no rescue was obtained with ergosterol or cholesterol alone.

**Discussion**

This study is the first to report an effect of the major cholesterol-lowering drugs, simvastatin and atorvastatin, on the growth of pathogenic yeast. Both simvastatin and atorvastatin caused strong inhibition of growth in four *Candida* species and *A. fumigatus*. This growth inhibition is likely to be due to lower levels of ergosterol in the cell caused by growth in the presence of simvastatin or atorvastatin. This has been demonstrated in previous studies with the related statin, lovastatin (also known as mevinolin), where growth inhibition and lowering of ergosterol levels were observed when the yeasts *Rhodotorula rubra* (Baranova et al., 1996), *S. cerevisiae* (Lorenz & Parks, 1990) and the mould *Tolypocladium inflatum* (Baranova et al., 1996; Bibikova et al., 2004) were grown in its presence. We have confirmed a decrease in ergosterol levels in simvastatin-treated *Candida* (unpublished data). Lower ergosterol levels are expected to arise as a result of inhibition of HMG-CoA reductase, a key enzyme in mevalonate biosynthesis. It is notable that rescue with ergosterol or cholesterol of the statin-induced growth inhibition in our studies has been incomplete. This could be due to sterol uptake rates being growth limiting or due to
competition. Alternatively, it should be noted that other effects might be expected, given the number of end products resulting from the mevalonate pathway or the effects on the membrane caused by ergosterol depletion. For example, reduced protein prenylation has been shown to be a major effect of lovastatin in *Mucor racemosus*, where reduced prenylation of Ras proteins results in apoptosis-like cell death (Roze & Linz, 1998). Experiments in rich media that contained an unspecified amount of ergosterol also demonstrated a statin-induced growth inhibition, albeit at higher statin levels, than required when ergosterol was absent.

The results indicate that statins may have a potential role as antifungal agents. In addition to the previously mentioned studies with lovastatin, Chin *et al.* (1997) found that lovastatin, pravastatin and simvastatin had no antifungal activity in an agar well drug diffusion assay. However, this may be due to a lack of activity of the prodrugs lovastatin and simvastatin. We found it necessary to activate simvastatin by hydrolysis to produce the active component that normally arises by metabolic alteration. It is likely that the statins used by Chin *et al.* (1997) were not being activated by hydrolysis to produce the active component. In addition, drug diffusion assays also depend on drugs being amenable to diffusion. Chin *et al.* (1997) showed that fluvasatin (which is not a prodrug) was an active antifungal against *Candida* species and against *Cryptococcus neoformans*. In addition, when fluvasatin was combined with fluconazole or itraconazole, substantially synergistic antifungal activities were demonstrated (Chin *et al.*, 1997), although another study failed to find synergy (Nash *et al.*, 2002). It would appear that there may be potential benefits in new antifungal therapies when considering statins alone and in combination with existing antifungal agents.

Our work shows that simvastatin and atorvastatin, and we suggest other statins too, may be effective as antifungal agents, although our work indicates that *C. krusei* is refractory to these statins. It is of interest that *C. krusei* displays insensitivity to the azole drugs that also target ergosterol synthesis (Orozco *et al.*, 1998). The levels of statins required for inhibition in our studies are similar to the levels used in the treatment of hypercholesterolaemia (Corsini *et al.*, 1999). A complicating factor in treatment of systemic infections with statins, however, would be the high levels of cholesterol. For example, a typical level of serum cholesterol is 2 mg mL\(^{-1}\) (5 mM) and we have demonstrated that levels as low as 12 μg mL\(^{-1}\) cholesterol can cause a 50% reduction in the statin-induced growth inhibition. Where there is an absence of competing cholesterol, statins may be able to provide more benefit.

The widespread use of statins in the human population and their differential effects on fungal species may suggest that statins could alter the normal pattern of fungal colonization. Furthermore, if statin treatments led to lower ergosterol levels in fungi carried by a statin-treated individual, this would lead to altered sensitivity to the many antifungal drugs (e.g. amphotericin, fluconazole) that target ergosterol.

![Fig. 2. Aspergillus fumigatus: growth inhibition by statins and rescue of growth with sterols.](image)
or its production? We consider that our studies suggest a need for further investigations to answer these questions.

Acknowledgements

The provision of *Aspergillus fumigatus* and *Candida* strains by Dr Wieland Meyer and Dr John Warmington is gratefully acknowledged. We also thank Dr Paul Vaughan, Dr Anna Johnson and Dr Connie Darmanin for their helpful comments in the preparation of this manuscript.

References


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