Lovastatin Has Significant Activity against Zygomycetes and Interacts Synergistically with Voriconazole

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Zygomycetes are emerging opportunistic molds resistant to most conventional antifungals. We evaluated the in vitro activity of lovastatin (LOV), a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor, against seven clinical isolates of Zygomycetes by using standard microdilution methods in three different media, disk diffusion testing, and viability dye staining. To further study the in vivo efficacy of LOV against zygomycetes, we developed a Drosophila melanogaster model of zygomycosis. In different experiments, groups of Toll-deficient (Tl) flies fed LOV-containing food were subsequently injected with two representative Zygomycetes isolates (Mucor and Rhizopus spp.). Finally, we examined the effects of LOV on voriconazole (VRC) activity against zygomycetes in vitro by checkerboard dilution, Epsilometer test-based methods, and bis-(1,3-dibutylbarbituric acid) trimethine oxonol staining and in vivo in Tl flies fed food containing LOV plus VRC and infected with zygomycetes. LOV exhibited significant, medium, and strain-independent fungicidal activity against all Zygomycetes isolates in vitro by all testing methods (MIC90 48.0 μg/ml; 50% minimal fungicidal concentration, 56.0 μg/ml; 50% effective concentration, 29.4 μg/ml [6.6 to 38.9 μg/ml]). Tl flies fed LOV-containing food and infected with Mucor had a significantly better 6-day survival rate than did infected Tl flies fed regular food (P = 0.0005). LOV displayed in vitro synergy with VRC against all Zygomycetes isolates (fractional inhibitory concentration index, 0.104 to 0.290) by all methods used. LOV also displayed synergy with VRC in the Drosophila model of zygomycosis (P < 0.01). LOV is significantly active against zygomycetes and synergizes with triazoles inherently resistant to them, such as VRC. The clinical significance of these findings needs to be further explored.

Fungi of the class Zygomycetes, order Mucorales, have been increasingly reported to cause opportunistic infections in a variety of immunocompromised hosts (11, 28). Rhizopus species cause more than 70% of Zygomycetes infections, whereas Mucor, Absidia, Rhizomucor, and Cunninghamella species are less frequently encountered pathogens (11, 16, 28). Importantly, zygomycosis has a particularly unfavorable outcome (11, 16, 28) because of delayed diagnosis as well as the inherent resistance of zygomycetes against most conventional antifungal agents (6, 11, 28, 30). Besides amphotericin B (AMB), only the investigational triazole posaconazole has shown promising activity against zygomycetes (6, 11, 28, 30, 32). Therefore, there is a dire need for development of novel treatment strategies against zygomycosis.

Lovastatin (LOV) is a statin that acts by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which is the rate-limiting enzyme of the mevalonate pathway (26). LOV as well as other statins has activity against a variety of pathogens both in vitro and in vivo (12, 13, 31). Importantly, statins have been shown to have antifungal activity in vitro against both the nonpathogenic yeast Saccharomyces cerevisiae and the pathogenic yeasts Candida spp. and Cryptococcus neoformans (5, 22). In a recent study, LOV induced apoptosis-like cell death in a Mucor racemosus isolate at relatively high concentrations (29). Moreover, statins have been reported to exhibit synergistic interactions with azoles against Candida, even against azole-resistant Candida isolates (5).

In the present study, we evaluated the activity of LOV against a range of clinically important Zygomycetes spp. in vitro by using independent susceptibility methods. We also tested in a Drosophila melanogaster model of zygomycosis the efficacy of LOV in vivo against two representative Zygomycetes isolates (21). We further evaluated whether LOV and voriconazole (VRC), a triazole with an inherent lack of activity against Zygomycetes spp. (6, 11, 30), have synergistic effects in vitro and in vivo (Drosophila model) against zygomycetes. Both these pilot in vitro and in vivo studies against zygomycetes demonstrated that LOV has significant, broad-spectrum fungicidal activity and synergy with VRC.

MATERIALS AND METHODS

Zygomycetes isolates. We used four Rhizopus isolates (two Rhizopus homothallic isolates and two Rhizopus oryzae isolates), two Mucor circinelloides isolates, and one Cunninghamella bertholletiae isolate collected from patients with cancer who had zygomycosis. We confirmed genus identification of the Zygomycetes isolates as described previously (33). Candida parapsilosis strain ATCC 22019 was used for quality control purposes in all in vitro experiments.

Drug stock solutions. LOV (meviltonin) was kindly provided by Merck (Merck, Sharp, and Dohme Research Laboratories, Rahway, N.J.). LOV was hydrolyzed in active open acid form as described previously (9), and a stock solution (4 μg/ml in a 10% alcohol sterile solution) was stored at −20°C until use. Additionally, AMB deoxycholate (Sigma Chemical Co., St. Louis, Mo.), itraconazole (ITC) (Janssen Pharmaceutica, Titusville, N.J.), VRC (Pfizer, Inc., New York, N.Y.), and caspofungin (CAS) (Merck, Rahway, N.J.) were obtained in
TABLE 1. Susceptibilities of the seven clinical isolates of *Zygomycetes* spp. to the antifungal agents tested in RPMI medium\(^a\)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC/MFC (µg/ml)</th>
<th>AMB</th>
<th>ITC</th>
<th>VRC</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. bertholletiae</em> 506313</td>
<td>1/2.0/2.0</td>
<td>2/4</td>
<td>8/&gt;16</td>
<td>32/32</td>
<td></td>
</tr>
<tr>
<td><em>R. homothallicus</em> 541783</td>
<td>0.5/1.0</td>
<td>1/4</td>
<td>8/&gt;16</td>
<td>32/32</td>
<td></td>
</tr>
<tr>
<td><em>R. oryzae</em> 518749</td>
<td>0.5/1.0</td>
<td>1/4</td>
<td>8/&gt;16</td>
<td>32/32</td>
<td></td>
</tr>
<tr>
<td><em>R. homothallicus</em> 529120</td>
<td>0.5/1.0</td>
<td>2/16</td>
<td>8/&gt;16</td>
<td>32/32</td>
<td></td>
</tr>
<tr>
<td><em>M. circinelloides</em> 424760</td>
<td>0.5/1.0</td>
<td>4/16</td>
<td>8/&gt;16</td>
<td>32/32</td>
<td></td>
</tr>
<tr>
<td><em>M. circinelloides</em> 488128</td>
<td>0.5/1.0</td>
<td>4/16</td>
<td>8/&gt;16</td>
<td>32/32</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) CLSI broth microdilution method M-38A.


d of the drugs ranged from 2.00 to 64.00 µg/ml for LOV and 0.5 to 64.00 µg/ml for VRC. Wells were subsequently inoculated with 100 µl of a standardized conidial suspension of each *Zygomycetes* isolate (final concentration, 0.4 × 10¹⁰ to 5.0 × 10¹⁰ conidia/ml), and trays were incubated for 22 h at 35°C. Next, 50 µl of the XTT solution was added to each well, the tray was incubated for an additional 2 h, and formazan absorbance was determined as described above. The fraction inhibitory concentration (FIC) of each drug was calculated by dividing the MIC of the drug when used in combination by its MIC when used alone. FIC values then were added together to define the interaction of the combination (19). Synergy was defined as an FIC of ≤0.5, whereas antagonism was defined as an FIC > 4; off-scale MICs were raised to the next highest MIC. Control wells containing medium alone were included in each experiment. All experiments were performed in triplicate.

**DIBAC mortality staining of *Zygomycetes* isolates.** Staining with the fluorescein di-bis-(1,3-diethylbarbituric acid) trimethine oxonol (DIBAC) was performed as described previously by Bowman et al. (3). Briefly, conidia from representative *Zygomycetes* isolates (one each of *C. oryzae*, *M. circinelloides*, and *C. bertholletiae*) were suspended in RPMI medium at a final concentration of 4 × 10¹⁰ to 5.0 × 10¹⁰ conidia/ml and incubated for 12 h at 35°C to generate hyphae. Aliquots of the hyphae were then mixed with each drug to produce the desired final concentration of LOV alone (4 × 50% effective concentration [EC₅₀]) and VRC alone (2 µg/ml). VRC in combination with LOV (2 and 4 µg/ml, respectively), and AMB (2 µg/ml). After incubation at 35°C for an additional 6 h, the hyphae were washed twice in MOPS (pH 7) buffer solution. DIBAC stain (final concentration, 2 µg/ml) was added accordingly, and samples were incubated for 1 h at room temperature in the dark with shaking (3). Samples were then washed twice again with MOPS, pH 7, and resuspended for photomicrography. Photomicrographs of the hyphae were taken under a triple-band fluorescent microscope (Olympus BX-51; Olympus, Melville, N.Y.) as described previously (3). Study of in vitro synergy of LOV and VRC by agar dilution and Epsilometer testing methods. To evaluate the effects of LOV on VRC activity against *zygomycetes*, we used a strategy combining the Epsilometer test (Etest; AB Biodisk, Solna, Sweden) and agar dilution susceptibility methods as described previously.

**TABLE 2. MICs and MFCs of LOV against the seven clinical isolates of *Zygomycetes* spp. in RPMI, RPMI-2, and YNB media\(^a\)**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>RPMI MIC/MFC</th>
<th>EC₅₀</th>
<th>Hill slope</th>
<th>RPMI-2 MIC/MFC</th>
<th>EC₅₀</th>
<th>Hill slope</th>
<th>YNB MIC/MFC</th>
<th>EC₅₀</th>
<th>Hill slope</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. bertholletiae</em> 506313</td>
<td>40/481</td>
<td>33.9 ± 6.6</td>
<td>-2.3 ± 1.1</td>
<td>32/40</td>
<td>7.3 ± 0.9</td>
<td>-4.9 ± 7.2</td>
<td>32/40</td>
<td>28.6 ± 5.4</td>
<td>-6.4 ± 7.0</td>
</tr>
<tr>
<td><em>R. homothallicus</em> 541783</td>
<td>56/56</td>
<td>38.9 ± 7.2</td>
<td>-4.7 ± 3.7</td>
<td>56/56</td>
<td>55.8 ± 52.0</td>
<td>-1.5 ± 2.2</td>
<td>48/64</td>
<td>30.6 ± 27.2</td>
<td>-1.2 ± 2.3</td>
</tr>
<tr>
<td><em>R. oryzae</em> 518749</td>
<td>48/48</td>
<td>27.1 ± 13</td>
<td>-3.3 ± 5.9</td>
<td>40/48</td>
<td>20.4 ± 12.5</td>
<td>-1.3 ± 1.6</td>
<td>32/40</td>
<td>19.2 ± 10.3</td>
<td>-1.5 ± 1.0</td>
</tr>
<tr>
<td><em>R. homothallicus</em> 529120</td>
<td>48/56</td>
<td>20.2 ± 3.1</td>
<td>-4.0 ± 2.4</td>
<td>56/56</td>
<td>17.7 ± 2.6</td>
<td>-4.3 ± 3.0</td>
<td>40/64</td>
<td>19.4 ± 4.0</td>
<td>-1.9 ± 0.9</td>
</tr>
<tr>
<td><em>R. oryzae</em> 557969</td>
<td>56/64</td>
<td>32.9 ± 2.4</td>
<td>-5.5 ± 1.9</td>
<td>48/64</td>
<td>35.9 ± 11.6</td>
<td>-1.2 ± 0.7</td>
<td>56/64</td>
<td>11.3 ± 9.7</td>
<td>-0.8 ± 0.7</td>
</tr>
<tr>
<td><em>M. circinelloides</em> 424760</td>
<td>32/32</td>
<td>6.6 ± 2.5</td>
<td>-1.2 ± 0.6</td>
<td>16/24</td>
<td>6.2 ± 2.5</td>
<td>-1.8 ± 1.5</td>
<td>24/24</td>
<td>9.8 ± 3.5</td>
<td>-2.4 ± 2.3</td>
</tr>
<tr>
<td><em>M. circinelloides</em> 488128</td>
<td>56/59</td>
<td>29.4 ± 4.4</td>
<td>-3.8 ± 2.3</td>
<td>48/48</td>
<td>27.0 ± 3.0</td>
<td>-4.4 ± 1.8</td>
<td>40/40</td>
<td>24.7 ± 6</td>
<td>-2.4 ± 1.3</td>
</tr>
</tbody>
</table>

\(^a\) CLSI broth microdilution method M-38A.

\(^b\) CI, confidence interval.
We performed Etest susceptibility testing of VRC against each *Zygomycetes* isolate in RPMI plates containing a standard noninhibitory concentration of LOV (4 \( \mu \)g/ml) (7). As controls, we determined VRC MICs against *Zygomycetes* by using the Etest method on regular RPMI plates (without LOV). We read the VRC MIC at 24 h as the drug concentration at the point where the growth ellipse intersected the strip (7) and, accordingly, statistically compared the changes in VRC MICs (15). All MIC determinations were carried out in triplicate on different days; the median 24-h MICs were reported.

**Drosophila infection model.** In different experiments, we infected Toll-deficient flies (Tl flies) (2- to 4-day-old female flies, 30 per experimental group) with two representative *Zygomycetes* isolates (*M. circinelloides* 424760 and *R. oryzae* 557969). We injected the thoraxes of Tl flies with a thin sterile needle that had been dipped into a concentrated solution (10^8 conidia/ml) of each *Zygomycetes* isolate, as described previously (17, 20). After injection, we housed the flies at 29°C and transferred them to fresh vials every 2 days. We assessed survival daily until day 6 after injection. We performed each experiment at least in triplicate on different days.

**Drug protection experiments.** For assessment of LOV protection against lethal infection by each *Zygomycetes* isolate, different groups of Tl flies (30 per experimental group) were housed in empty vials for 6 to 8 h to starve and then transferred into vials containing LOV-mixed fly food (10 mg/ml) as described previously (2, 20). After 48 h, each group of Tl flies was infected with the
corresponding Zygomycetes isolate by injection and transferred daily into fresh LOV-containing vials at 29°C for 6 days. Flies that were starved for 6 to 8 h, transferred to vials containing regular fly food (without LOV), infected, and maintained in regular vials were used as controls. LOV protection was assessed daily until day 6 after infection. For the combination drug experiments, vials containing VRC alone (1 mg/ml), LOV alone (10 mg/ml), or VRC plus LOV (1 and 10 mg/ml, respectively) were prepared as described previously (20). Each experiment was performed at least in triplicate on different days.

Statistical analysis. Median MIC and MFC values were calculated based on experiments performed in triplicate. The Mann-Whitney U test or Kruskal-Wallis one-way analysis of variance with Dunn’s test was used when appropriate to assess significant differences in the corresponding MICs and MFCs. Survival curves were plotted by using Kaplan-Meier analysis, and differences in survival rates between the groups were analyzed by using the log rank test. A four-parameter logistic regression model (Hill equation) was fitted to XTT reduction assay data to determine EC50 values and the steepness of inhibitory dose-response curves (Hill slope) with the use of a curve-fitting software program (Prism 4; GraphPad Software, Inc., San Diego, Calif.). P values of less than 0.05 were considered statistically significant.

RESULTS

LOV has significant in vitro activity against Zygomycetes isolates. The MICs and MFCs for AMB, ITC, VRC, and CAS are listed in Table 1. AMB exhibited fungicidal activity against all of the Zygomycetes isolates tested, as the AMB MICs and MFCs (MIC50, 0.5 µg/ml; MFC50, 1 µg/ml) were equal or differed by less than 1 serial dilution in every case. By comparison, only ITC (MIC50, 4 µg/ml; MFC50, 8 µg/ml) demonstrated limited fungistatic efficacy against some Zygomycetes isolates, whereas neither VRC (MIC50, 8 µg/ml; MFC50, 16 µg/ml) nor CAS (MIC50, 32 µg/ml; MFC50, 32 µg/ml) had any activity at the concentrations tested.

The MICs and MFCs of LOV against Zygomycetes isolates in RPMI, RPMI-2, and YNB media are shown in Table 2. LOV exhibited fungicidal activity against all of the isolates (MIC50, 48 µg/ml [range, 32 to 56 µg/ml]; MFC50, 56 µg/ml [range, 48 to 64 µg/ml]) in all media tested. Although there were no significant interspecies differences, there was a trend towards improved activity of LOV against Mucor species.

The activity of LOV against zygomycetes was also clearly observable with disk diffusion susceptibility testing (Fig. 1A). Notably, LOV produced a clear zone of inhibition of growth against all Zygomycetes isolates (mean radius ± standard deviation for LOV, 12.570 ± 3.861 mm).

LOV results in significant, concentration-dependent reduction of Zygomycetes biomass by the XTT assay. We found a linear relationship between XTT reduction to formazan (the colorimetrically assayed product) and starting inoculum of zygomycetes for all of the isolates tested over a range of inocula (Fig. 2A). Therefore, a reduction of the formazan absorbance from 3.0 to ≤0.5 optical density as a result of LOV activity against zygomycetes correlated with an approximately-2.5-log reduction in hyphal biomass.

LOV exhibited potent antifungal activity against all of the Zygomycetes isolates (median EC50, 29.4 µg/ml [range, 6.6 to 38.9 µg/ml]) in a steep concentration–inhibitory-effect curve (Hill slope range, −1.2 to −4.7). A substantial reduction (≥2.5 log) of the hyphal biomass was evident for all of the isolates as the LOV concentration approached the MFC (Fig. 2B). Again, LOV appeared to be more effective against Mucor spp. than against Rhizopus and Cunninghamamella spp. The in vitro activity of LOV was consistent among all three media tested (Table 2).
LOV has fungicidal effects against Zygomycetes hyphae by DiBAC staining. While control hyphae cells (without drug pre-exposure) demonstrated absence of fluorescence after DiBAC staining, there was clearly evident Zygomycetes hyphal damage in high concentrations of LOV (4× EC₅₀, 16 μg/ml) of each isolate tested; at a subinhibitory LOV concentration (4 μg/ml), the fluorescence indicative of hyphal damage caused by LOV was minimal to absent (Fig. 3). AMB exhibited a pronounced...
hyphal damage at a concentration equal to the MFC (2 μg/ml), whereas VRC had no fungicidal activity at a clinically achievable concentration (2 μg/ml).

Protection of Tl flies infected with Zygomycetes by LOV. Injection of a concentrated solution of conidia (10⁸ conidia/ml) of either R. oryzae or M. circinelloides resulted in a hyperacute infection with equally high mortality rates in Tl flies (>80%) within 3 days of infection (Fig. 4A). LOV-fed flies had a significantly better survival rate 6 days after infection with M. circinelloides (50%) than did control flies (<15%; P = 0.0005) (Fig. 4A). However, LOV had no activity in Tl flies infected with R. oryzae (Fig. 4A).

LOV exhibits synergistic interaction with VRC in vitro when tested in combination against Zygomycetes isolates. By XTT-based checkerboard, the combination of both LOV and VRC was synergistic against all of the Zygomycetes isolates tested (FIC index of ≤0.5), with FIC indices ranging from 0.104 to 0.290 (Table 3). Importantly, the interaction of LOV with VRC resulted in MICs (VRC MIC₅₀, 1 μg/ml; LOV MIC₅₀, 2 μg/ml) that were in the range of clinically achievable concentrations of both drugs (Table 3).

LOV and VRC have significant synergistic effects against Zygomycetes by Etest/plate dilution method. The effects of LOV on VRC MICs against all of the Zygomycetes isolates tested by Etest/plate dilution method are shown in Table 3 and Fig. 1B. There was a significant, pronounced reduction in VRC MICs (threefold to sixfold) following concomitant exposure of all of the Zygomycetes isolates to both LOV and VRC (P < 0.0001).

LOV combination with VRC is fungicidal against Zygomycetes by DiBAC staining. The combination of VRC and LOV at subinhibitory concentrations for both drugs (2 and 4 μg/ml, respectively) potentiated fungicidal activity against Zygomycetes hyphae, as evidenced by DiBAC staining (Fig. 3). In pilot experiments, VRC alone was shown to cause minimal fluorescence indicative of cellular wall and membrane damage in Zygomycetes hyphae at concentrations up to 8 μg/ml. The effects of VRC combined with LOV on hyphal damage were comparable with those of AMB at concentrations equal to the MFC (2 μg/ml).

Protection of Tl flies infected with M. circinelloides by the combination of LOV and VRC. Previous studies showed that concentrations of VRC up to 2 mg/ml were not toxic in adult flies (21). We found that Tl flies fed with LOV plus VRC and infected with either R. oryzae or M. circinelloides had a significantly better survival rate than did infected flies fed with LOV alone (P < 0.01). VRC alone had no activity in infected Tl flies (Fig. 4B).

DISCUSSION

The development of oral antifungal therapeutic strategies is a major unmet medical need for zygomycosis, an opportunistic mycosis for which oral antifungal options are extremely limited (11). In the present study, we found that the in vitro susceptibilities of the Zygomycetes isolates to the antifungal drugs tested were in agreement with those from previous studies (6, 11, 30). Indeed, of the antifungal agents tested, only AMB exhibited significant fungicidal activity. Although it has been reported that Zygomycetes spp. might be susceptible to ITC in vitro (6), ITC exhibited marginal fungistatic activities against the isolates tested in our study.

However, the Rhizopus, Mucor, and Cunninghamella isolates that we tested were more resistant to ITC than were Absidia and Rhizomucor isolates tested elsewhere (6, 30). Our study also confirmed the well-described in vitro resistance of Zygomycetes spp. to VRC and CAS (6, 11, 30).
We also found that LOV had considerable in vitro fungicidal activity against all seven *Zygomycetes* isolates tested. Although we did not observe dramatic interspecies differences in susceptibility to LOV, the *Mucor* isolates appeared to be more susceptible to LOV than the other isolates were. In comparison, a recent study that used nonstandardized methods found that *Rhizomucor pusillus* strains were remarkably more sensitive to LOV than *Rhizomucor miehei* strains were according to agar diffusion susceptibility testing (23).

Furthermore, we observed that the activity of LOV against *Zygomycetes* isolates was consistent across several different culture media. Of note, there was a trend toward lower LOV MICs and EC_{50} values in YNB media. Our group and others have postulated that because enriched YNB medium facilitates fungal growth compared with RPMI and RPMI-2, it might result in increased metabolic activity of fungal cells and better drug penetration into the intracellular sites of drug action (20, 24).

To better characterize the effects of LOV against *Zygomycetes* spp., we employed a strategy combining different viability dye staining methods (18). We found that the XTT colorimetric assay was able to accurately quantify the fungicidal activity of fungal cells and better drug interaction by using DiBAC staining. Importantly, the concentrations of serum concentrations of LOV reported for mammalian models (2 to 20 μg/ml) and for humans (3.9 μg/ml) (4). Finally, we found that LOV and VRC exhibited synergistic effects against *Zygomycetes* isolates tested in vitro. We further verified this observation by using DiBAC staining. Importantly, the concentrations of LOV used in synergy studies with VRC were within the range of serum concentrations of LOV reported for mammalian models (2 to 20 μg/ml) or 0.5 to 5 μg/ml and for humans (3.9 μg/ml) (4). Finally, we found that LOV and VRC exhibited synergistic effects against two representative *Zygomycetes* isolates in vivo in the *Drosophila* model of zygomycosis. These results are in agreement with studies that reported in vitro synergy of statins to overcome azole resistance has been shown previously for *Candida* (5) and might have even greater clinical significance.

By employing checkerboard dilution and Etest-based methods, we found that at subinhibitory concentrations, LOV significantly potentiated the activity of VRC against all of the *Zygomycetes* isolates tested in vitro. We further verified this observation by using DiBAC staining. Importantly, the concentrations of LOV used in synergy studies with VRC were within the range of serum concentrations of LOV reported for mammalian models (2 to 20 μg/ml or 0.5 to 5 μg/ml) and for humans (3.9 μg/ml or 1 μg/ml) (4). Finally, we found that LOV and VRC exhibited synergistic effects against two representative *Zygomycetes* isolates in vivo in the *Drosophila* model of zygomycosis. These results are in agreement with studies that reported in vitro synergy of statins with different azoles against *Candida* and *Cryptococcus* species (5). Importantly, there is also evidence of in vivo synergy of LOV and azoles against *Trypanosoma cruzi*, a protozoan parasite in which, similar to fungi, ergosterol is an essential cell membrane component (31).

Although sequential inhibition of sterol biosynthesis may be plausible (22), the mechanisms of the antifungal activity of LOV remain largely unexplored because of the pleiotropic effects of LOV in cellular metabolism. For example, LOV is a well-known inhibitor of protein isoprenylation, a highly conserved essential process for cell proliferation, differentiation,
and apoptosis in eukaryotes from fungi to humans (4, 10, 26) as well as in prokaryotes (34). Importantly, LOV was recently shown to induce apoptosis of several human cancer cell lines by mechanisms involving inhibition of Ras and several other isoprenoids (1, 4, 13, 14, 26). Similarly, LOV was recently shown to suppress the expression of three Ras genes in *M. racemosus* and to induce apoptosis-like cell death (29). However, the exact mechanisms that mediate apoptosis in fungi seem to be complex and have not been elucidated. Exploring the underlying mechanisms of LOV activity against *Zygomycetes* might pave the way for the development of compounds with more selective action. This is particularly important in view of the fact that significant toxicity has been reported as a result of statin interactions with azoles or other drugs that are metabolized in the liver by cytochrome P450 (4). Finally, expanding these observations to other members of the statin family with or without other azoles that exhibit activity against *Zygomycetes* (e.g., posaconazole) or other classes of antifungal agents would be of interest.

In conclusion, the significant antifungal activity of LOV against *Zygomycetes* as well as its synergy with triazoles may result in significant clinical applications in the near future.

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