# Interactions between statins and *Penicillium chrysogenum* antifungal protein (PAF) to inhibit the germination of sporangiospores of different sensitive *Zygomycetes*

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## Abstract

This study reports on the antifungal activities of statins combined with an antifungal compound secreted by *Penicillium chrysogenum*, PAF. Several species belonging in the class *Zygomycetes* are considered to be agents of human or animal mycoses; other species have significance as postharvest plant pathogens. In the present work, four species (*Rhizopus stolonifer*, *Mortierella wolfii*, *Syncephalastrum racemosum* and *Mycotypha africana*) that exhibited different sensitivities to lovastatin and PAF in previous experiments were investigated. The efficiencies with which four statins (lovastatin, simvastatin, rosuvastatin and atorvastatin) inhibited sporangiospore germination in the absence or in the presence of a constant concentration of PAF were studied. PAF and lovastatin acted synergistically on the sporangiospore germination of *Mycotypha africana*, and similar effects of the combinations PAF-rosuvastatin and PAF-atorvastatin were observed on *S. racemosum*.

## Introduction

A number of members of the class *Zygomycetes* are important as postharvest pathogens of agricultural products; *Rhizopus*, *Mucor* and *Gilbertella* species are among the most frequently isolated causative agents of fungal rots (Takó & Csernetics, 2005). Other representatives of this group are known to be opportunistic pathogens of humans and animals. The incidence of zygomycoses has increased continuously during recent years as a consequence of poorly controlled diabetes mellitus, haematologic malignancy, solid organ or bone marrow transplantation, steroid use, metabolic acidosis, deferoxamine therapy and severe and prolonged neutropenia (Ribes *et al.*, 2000). Treatment with amphotericin B is the standard and, in fact, the only available effective therapy. However, it is quite toxic and has side effects: it causes fever, rigours and chills, and can also seriously damage the kidneys (Gallagher *et al.*, 2003; Vicente *et al.*, 2003).

There is therefore a substantial demand for new types of compounds with antifungal activity. Statins and proteins with similar structure like defensins secreted by some filamentous fungi are interesting in this respect, as they have effective inhibitory potential as concerns both the hyphal extension and the germination of the sporangiospores.

Statins are fungal metabolites (mevastatin, lovastatin, simvastatin and pravasatin) or fully synthetic compounds (atorvastatin, cerivastatin, fluvastatin, pitavastatin and rosuvastatin). They are known to be competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyses the conversion of HMG-CoA to mevalonate, a rate-limiting step in the isoprenoid biosynthetic pathway. The effects of statins are therefore connected to the inhibition of the synthesis of important isoprenoids, e.g. farnesyl pyrophosphate and geranylgeranyl pyrophosphate. These intermediates are important lipid attachments for the γ subunit of heterotrimeric G-proteins (Liao & Laufs, 2005), guanosine triphosphate-binding protein Ras and Ras-like proteins (Rho, Rab, Rac, Ral or Rap) (Liao, 2002; Ghittoni *et al.*, 2005; Liao & Laufs, 2005); hence, statins act as inhibitors of some G-protein actions and Ras or Ras-like signalling (Cordle *et al.*, 2005).

Statins have been shown to exert antifungal activity against the pathogenic yeasts *Candida* spp. and *Cryptococcus neoformans* and the nonpathogenic *Saccharomyces cerevisiae* (Lorenz & Parks, 1990; Chin *et al.*, 1997). In a recent study, the abilities of atorvastatin and simvastatin to inhibit the

growth of *Candida* species and *Aspergillus fumigatus* were tested (Macreadie *et al.*, 2006); the statins strongly inhibited the growth of all species, except *Candida krusei*. In another study, lovastatin (at relatively high concentrations) induced apoptosis-like cell death in *Mucor circinelloides* (Roze & Linz, 1988). The inhibitory effect of lovastatin has been studied on two opportunistic pathogenic *Zygomycetes*: *Rhizomucor pusillus* and *Rhizomucor miehei*. A medium supplemented with 6 μg lovastatin $mL^{-1}$ was effective in reducing the hyphal extension of *Rhizomucor miehei* and delayed the germination of sporangiospores of *Rhizomucor pusillus* (Lukács *et al.*, 2004). The antifungal effects of lovastatin and its synergism with voriconazole against seven clinical isolates of different *Zygomycetes* (e.g. *Mucor circinelloides*, *Rhizopus homothallicus*, *Rhizopus oryzae* and *Cunninghamella bertholletiae*) were recently tested with several methods (Chamilos *et al.*, 2006): lovastatin exhibited significant, medium- and strain-independent fungicidal activity against all *Zygomycetes* isolates and displayed *in vitro* synergy with voriconazole against all tested strains.

The antifungal proteins secreted by some filamentous fungi are effective inhibitors of hyphal extension and spore germination. The features of these proteins are a low molecular mass (5.8–6.6 kDa), a basic character and the presence of 6–8 cysteine residues and several disulphide bonds. Proteins with such properties have been isolated and investigated from four fungal species (*Penicillium chrysogenum*, *Penicillium nalgiovense*, *Aspergillus giganteus* and *Aspergillus niger*); furthermore, *in silico* investigation of genomic databases has revealed a putative protein with high homology to *P. chrysogenum* antifungal protein (PAF) in *Gibberella zeae* (Marx, 2004). It has been shown that, in sensitive fungi, PAF is localized intracellularly and exerts multiple detrimental effects: induction of morphological changes, membrane perturbation and intracellular oxidative stress (Kaiserer *et al.*, 2003). Among such proteins, only the effects of PAF have been studied against *Zygomycetes* (some of them are considered to be opportunistic pathogens). The *Mucor*, *Actinomucor*, *Cokeromyces*, *Gilbertella*, *Rhizopus*, *Saksanea* and *Syncephalastrum* strains tested were insensitive to PAF, independent of the culture conditions and inhibition tests applied. On the other hand, *Absidia*, *Micromucor*, *Mortierella*, *Mycotypha*, *Rhizomucor*, *Rhizopus*, *Thamnostylum* and *Umbellopsis* proved to be sensitive to PAF. The PAF sensitivities of some *Zygomycetes* depended on the culture medium and the type of inhibition test applied (e.g. for *Mycotypha africana*, *Rhizomucor pusillus* and *Umbellopsis isabellina*, the inhibitory effect on hyphal growth was usually less than that on sporangiospore germination) (Galgóczy *et al.*, 2005).

In the present study, the effects of combinations of four different statins (lovastatin, simvastatin, rosuvastatin and atorvastatin) with PAF against four *Zygomycetes* (*Rhizopus stolonifer*, *Mortierella wolfii*, *Syncephalastrum racemosum* and *Mycotypha africana*) were tested *in vitro*. The strains involved were selected on the basis of their different susceptibilities to lovastatin (unpublished observation) and PAF (Galgóczy *et al.*, 2005) detected in previous tests.

## Materials and methods

### Strains and media

The effects of the statins and PAF on four fungal isolates (*Rhizopus stolonifer* SZMC 11101, *Mortierella wolfii* NRRL 28640, *S. racemosum* SZMC 2011 and *Mycotypha africana* NRRL 2978), representing four different sensitivity groups, were examined. The fungal strains were maintained on malt extract slants (0.5% malt extract, 0.5% yeast extract, 0.5% glucose, 1.0% $KH_2PO_4$, 1.5% agar) at 4 °C. The tests of inhibition of sporangiospore germination and hyphal growth were performed in yeast extract–peptone–glucose medium (SPEC; 0.1% yeast extract, 0.05% peptone, 2.0% glucose).

### Purification of PAF

PAF was purified in a modification of the method described by Marx *et al.* (1995). Briefly, *P. chrysogenum* Q176 was grown in a sucrose (20 g $L^{-1}$) – $NaNO_3$ (3 g $L^{-1}$) minimal medium for 96 h at 25 °C with shaking (220 r.p.m.) (Marx *et al.*, 1995). After harvesting of the mycelia by centrifugation and separation of the low-molecular-weight protein fraction in Amicon Stirred Cells (V = 50 mL, Biomix PBTK ultrafiltration discs, size exclusion limit $M_r$ = 30 000; Millipore), PAF was purified by ion-exchange chromatography on a CM Sephadex Fast Flow column (2 × 18 cm, equilibrated with 50 mM sodium phosphate buffer, pH = 6.6, flow rate 1 mL $min^{-1}$, $t$ = 4 °C; Amersham-Pharmacia). PAF was eluted with a NaCl gradient (0.05–1.0 M) prepared in the equilibrating buffer. The quality of the PAF preparation was always checked with sodium dodecyl sulfate-polyacrylamide gel electrophoresis on precast Novex 16% Tris/Glycine gels (Invitrogen). Protein bands were visualized with Coomassie Brilliant Blue R staining. The purified protein was prepared in 25 mM Tris/HCl, pH = 7.5 and diluted in 150 μL SPEC medium without or with statins for antifungal experiments.

### Antifungal activity assays

Each of the statins, lovastatin (Mevacor, Merck Sharp & Dohme), rosuvastatin (Crestor, Merck Sharp & Dohme), simvastatin (Vasilip, Egis) and atorvastatin (Atorvox, Richter), was of pharmaceutical grade. The *in vitro* antifungal activities of the statins, PAF and their combinations were determined in 96-well microtitre plate bioassays by measuring the absorbances of fungal cultures at 620 nm. Briefly, 150 μL of various concentrations of statins with or without

PAF in SPEC medium were mixed with 50 μL of sporangiospore suspension ($10^5$ spores $mL^{-1}$). The final concentrations were 1, 2, 4, 8, 16, 32, 64 and 128 μg $mL^{-1}$ of statins. If PAF was added, its final concentration was 50 μg $mL^{-1}$. The applied concentration of PAF was chosen on the basis of an earlier study (Galgóczy *et al.*, 2005), where the effect of the PAF on the rate of sporangiospore germination was tested on different solid media. The plates were incubated for 24 h at 30 °C, and the absorbances were then measured with a microtitre plate reader (ASYS Jupiter HD–ASYS Hitech). The fresh medium was used as a background for the spectrophotometric calibration. For calculation of the inhibition rates, the absorbances of the untreated control cultures were referred to 100% of growth, in each case. The interaction ratio (IR) between the antifungal agents was calculated by making use of the Abbott formula: $I_e = X + Y - (XY/100)$, where $I_e$ is the expected percentage inhibition for a given interaction, and $X$ and $Y$ are the percentage growths inhibited by the compounds used alone. If $I_0$ is the observed percentage inhibition, the IR is given by $IR = I_0/I_e$, which corresponds to the nature of the interaction between the antifungal compounds. When IR is between 0.5 and 1.5, the interaction is additive, IR > 1.5 denotes synergism and IR < 0.5 denotes antagonism interaction respectively (Moreno *et al.*, 2003). The experiments were repeated three times.

## Results

### Sensitivity to PAF

In previous tests, sporangiospore germination of *Rhizopus stolonifer* and *S. racemosum* was not affected by PAF, while *Mortierella wolfii* and *Mycotypha africana* proved to be sensitive as their spore germination was inhibited by 50–75% and 75–100%, respectively (Galgóczy *et al.*, 2005). However, the sensitivities of some *Zygomycetes* to PAF depended on the culture media and the selected inhibition tests. To allow calculation of IR for PAF and the statins, the PAF sensitivities of the strains were tested again in microtitre plate bioassays: 50 μg $mL^{-1}$ PAF decreased the growth rates of *Mortierella wolfii*, *Mycotypha africana* (sensitive species) and *S. racemosum* (slightly sensitive) to 65% (±3.3%), 72% (±8.3%) and 85% (±5.9%), respectively, whereas *Rhizopus stolonifer* proved to be completely resistant, similar to the results of the above-mentioned study.

### Sensitivity to the statins

The antifungal effects of the different statins varied: *Rhizopus stolonifer* and *Mortierella wolfii* were insensitive to lovastatin, even at higher concentrations. *Mycotypha africana* was moderately sensitive to this compound: the growth rate decreased evenly with increasing concentration

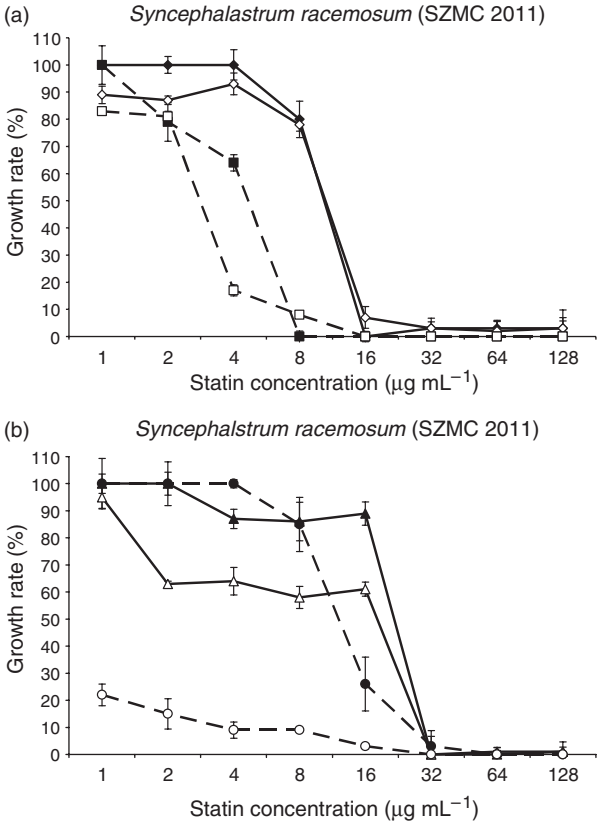


**Fig. 1.** Effects of lovastatin, simvastatin, rosuvastatin, atorvastatin and their combinations with 50 μg $mL^{-1}$ of PAF on the growth rate of *Syncephalastrum racemosum*. 85% (±5.9%) growth in the presence of 50 μg $mL^{-1}$ of PAF. (a) ◆, lovastatin; ◇, lovastatin +50 μg $mL^{-1}$ of PAF; ■, simvastatin; □, simvastatin +50 μg $mL^{-1}$ of PAF. (b) ▲, rosuvastatin; △, rosuvastatin +50 μg $mL^{-1}$ of PAF; ●, atorvastatin; ○, atorvastatin +50 μg $mL^{-1}$ of PAF.

of lovastatin, but it still remained almost 34% (±0.5%) at 128 μg $mL^{-1}$. *Syncephalastrum racemosum* was sensitive to lovastatin: 16 μg $mL^{-1}$ blocked its growth completely (Fig. 1a).

Simvastatin was active against *S. racemosum* and *Mycotypha africana*. The germination of *S. racemosum* spores was blocked by ≥8 μg $mL^{-1}$ (Fig. 1a). Although simvastatin inhibited the growth of *Mycotypha africana*, complete blockade was not achieved even at 128 μg $mL^{-1}$ (Fig. 2a). At ≥32 μg $mL^{-1}$, inhibition was observed in the case of *Rhizopus stolonifer* (55% (±1%) growth inhibition at 128 μg $mL^{-1}$), while *Mortierella wolfii* proved completely resistant.

Rosuvastatin and atorvastatin were active against the susceptible strains in similar concentrations. The minimal concentrations of rosuvastatin and atorvastatin that blocked spore germination were 64 μg $mL^{-1}$ for *Rhizopus stolonifer*, 32 μg $mL^{-1}$ for *S. racemosum* and 8 μg $mL^{-1}$ for *Mycotypha africana*, respectively (Figs 1b and 2b). *Mortierella wolfii* was

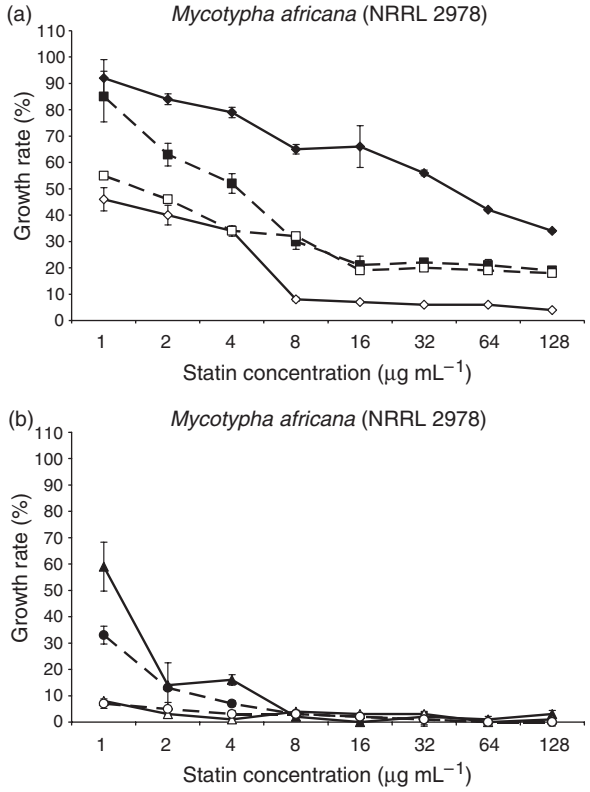


**Fig. 2.** Effects of lovastatin, simvastatin, rosuvastatin, atorvastatin and their combinations with 50 μg mL$^{-1}$ of PAF on the growth rate of *Mycotypha africana*. 72% (±8.3%) growth in the presence of 50 μg mL$^{-1}$ of PAF. (a) ◆, lovastatin; ◇, lovastatin +50 μg mL$^{-1}$ of PAF; ■, simvastatin; □, simvastatin +50 μg mL$^{-1}$ of PAF. (b) ▲, rosuvastatin; △, rosuvastatin +50 μg mL$^{-1}$ of PAF; ●, atorvastatin; ○, atorvastatin +50 μg mL$^{-1}$ of PAF.

the least sensitive: 128 μg mL$^{-1}$ decreased its growth rate to 41% (±5.8%) and 68% (±3.8%), respectively.

### Interaction between PAF and statins

In this study, PAF was applied at a constant concentration (50 μg mL$^{-1}$), combined with eight different concentrations of each statin.

Interactions were not found in *Rhizopus stolonifer*. This species was completely insensitive to both lovastatin and PAF. The inhibitions induced by the combinations simvastatin-PAF, rosuvastatin-PAF and atorvastatin-PAF were the same as those observed for the statins alone.

*Mortierella wolfii* was practically insensitive to the applied statins. The *c.* 40% decrease in the growth of *Mortierella wolfii* in the presence of the statin-PAF combinations corresponds to the activity of PAF applied without a statin.

For *S. racemosum*, no interaction between lovastatin and PAF was detected: there was no significant difference in the sensitivity to lovastatin or lovastatin-PAF (Fig. 1a). Simvastatin at 4 μg mL$^{-1}$ acted synergistically (IR = 1.82) with PAF, decreasing the growth rate to 17% (±2%). Higher concentrations of simvastatin added together with PAF led to the total blockade of growth (Fig. 1a). Rosuvastatin acted synergistically with PAF even at low statin concentrations (≥2 μg mL$^{-1}$) (IR = 2.47), but it is worth mentioning that rosuvastatin alone can evoke a complete growth inhibition at ≥32 μg mL$^{-1}$ concentrations (Fig. 1b). Atorvastatin and PAF acted on *S. racemosum* synergistically at 1–8 μg mL$^{-1}$ of atorvastatin (IRs = 5.2, 5.67, 6.07 and 3.28), and additively at 16 μg mL$^{-1}$ of atorvastatin (IR = 1.25); higher statin concentrations combined with PAF resulted in complete growth inhibition (Fig. 1b).

*Mycotypha africana* was sensitive to all statins and PAF, and the inhibition increased when the two types of compounds were applied in combinations (Fig. 2). Synergistic interactions were detected between lovastatin (at 1–8 μg mL$^{-1}$) and PAF (IRs = 1.6, 1.52, 1.53 and 1.73). The addition of 8 μg mL$^{-1}$ lovastatin together with PAF reduced the growth rate to 8% (±0.8%); higher concentrations resulted in further slight decreases in the growth, but complete inhibition was not achieved. Simvastatin interacted additively with PAF at 1–4 μg mL$^{-1}$ (IRs = 1.16, 0.99 and 1.05). However, no effect of PAF was detected at higher simvastatin concentrations: the inhibition rates were the same as those with the same amounts of simvastatin without PAF (Fig. 2a). One μg mL$^{-1}$ of rosuvastatin and PAF acted synergistically (IR = 1.6) against *Mycotypha africana*, causing an inhibition of 92% (±0.2%). Additive interactions were observed at 2–4 μg mL$^{-1}$ (IRs = 1.08 and 1.18); higher rosuvastatin concentrations led to complete growth inhibition. Atorvastatin at 1–4 μg mL$^{-1}$ acted additively with PAF (IRs = 1.22, 1.05 and 1.02); higher concentrations in the presence of PAF inhibited germination completely (Fig. 2b). The inhibition rates obtained with the rosuvastatin-PAF and atorvastatin-PAF combinations were very similar.

Data of these experiments are summarized in Table 1.

## Discussion

The aim of this study was to investigate the possible interactions between statins and PAF against *Zygomycetes*. The tested strains represented different species that responded in distinct ways to the different statins and statin–PAF combinations. In general, most of the statins were effective to various extents against *Zygomycetes* and the antifungal activity could be increased by PAF supplementation. However, the synthetic statins (rosuvastatin and atorvastatin) proved to be more effective than the fungal metabolites (lovastatin and simvastatin). The inhibitory effects of these statins have not been investigated or detected previously in these fungi; data on the antifungal activity of statins on *Zygomycetes* have been available only as concerns

**Table 1.** Interactions between different statins and PAF (50 μg mL$^{-1}$) on the growth rate of *Syncephalastrum racemosum* and *Mycotypha africana*

| Statin (μg mL$^{-1}$) | $I_0$ | $I_e$ | IR | Type of interaction | Growth rate (%) |
|---|---|---|---|---|---|
| *Syncephalastrum racemosum* | | | | | |
| Simvastatin | | | | | |
| 4 | 83 | 45.6 | 1.82 | Synergistic | 17 |
| Rosuvastatin | | | | | |
| 2 | 37 | 15 | 2.47 | Synergistic | 63 |
| 4 | 36 | 13 | 2.77 | Synergistic | 64 |
| 8 | 42 | 26.9 | 1.56 | Synergistic | 58 |
| 16 | 39 | 24.35 | 1.6 | Synergistic | 61 |
| Atorvastatin | | | | | |
| 1 | 78 | 15 | 5.2 | Synergistic | 22 |
| 2 | 85 | 15 | 5.67 | Synergistic | 15 |
| 4 | 91 | 15 | 6.07 | Synergistic | 9 |
| 8 | 91 | 27.75 | 3.28 | Synergistic | 9 |
| 16 | 97 | 77.9 | 1.25 | Additive | 3 |
| *Mycotypha africana* | | | | | |
| Lovastatin | | | | | |
| 1 | 54 | 33.76 | 1.6 | Synergistic | 46 |
| 2 | 60 | 39.52 | 1.52 | Synergistic | 40 |
| 4 | 66 | 43.12 | 1.53 | Synergistic | 34 |
| 8 | 92 | 53.2 | 1.73 | Synergistic | 8 |
| 16 | 93 | 52.48 | 1.77 | Synergistic | 7 |
| 32 | 94 | 59.68 | 1.58 | Synergistic | 6 |
| 64 | 94 | 69.76 | 1.35 | Additive | 6 |
| 128 | 96 | 75.52 | 1.27 | Additive | 4 |
| Simvastatin | | | | | |
| 1 | 45 | 38.8 | 1.16 | Additive | 55 |
| 2 | 54 | 54.64 | 0.99 | Additive | 46 |
| 4 | 66 | 62.56 | 1.05 | Additive | 34 |
| Rosuvastatin | | | | | |
| 1 | 92 | 57.52 | 1.6 | Synergistic | 8 |
| 2 | 97 | 89.92 | 1.08 | Additive | 3 |
| 4 | 99 | 84 | 1.181 | Additive | 1 |
| Atorvastatin | | | | | |
| 1 | 93 | 76.24 | 1.22 | Additive | 7 |
| 2 | 95 | 90.64 | 1.05 | Additive | 5 |
| 4 | 97 | 94.96 | 1.025 | Additive | 3 |

lovastatin (Roze & Linz, 1988; Lukács *et al*., 2004; Chamilos *et al*., 2006). Natural statins caused complete inhibition at the tested concentrations only in the case of *S. racemosum*; the other species exhibited some degree of resistance against these statins, which, in certain cases, could be reduced with PAF. In a recent study, where the activity of lovastatin against seven clinical isolates from four *Zygomycetes* species was tested (Chamilos *et al*., 2006), significiant interspecies differences in susceptibility were not observed: all strains were sensitive to lovastatin, with MICs of 32–56 μg mL$^{-1}$. These results are not easily comparable with those of the present study, because the differences in the applied test method and the involved organisms. It is noteworthy that *Rhizopus oryzae* and *Rhizopus homothallicus* (reported to be sensitive to lovastatin) are closely related to *Rhizopus stolonifer*, which was found to be completely resistant in the present tests. Similar differences in the susceptibilities to lovastatin among the members of the genus *Rhizomucor* were observed by Lukács *et al*. (2004) in agar plate testing.

The antifungal activity of simvastatin against *Zygomycetes* was similar to that of lovastatin, with the exception of *S. racemosum*, which was the most sensitive to simvastatin.

Rosuvastatin and atorvastatin were the most effective against *Rhizopus stolonifer* and *Mycotypha africana*. Interestingly, these fungi were resistant to the synthetic statins: complete blockade of growth was not achieved even at 128 μg mL$^{-1}$. *Mortierella wolfii* proved to be the most resistant to the statins. None of the statins tested had significant effects on *Mortierella wolfii*, although rosuvastatin at 128 μg mL$^{-1}$ yielded an inhibition of 60%. The molecular background of the different levels of fungal resistance to statins is unknown; the assumption that it is connected to different copy numbers of the HMG-CoA reductase gene was not confirmed in *Rhizomucor* species (Lukács *et al*., 2004). The difference in the effectivity of the statins may also be explained in terms of their pharmacokinetic properties. Clinical studies have demonstrated that rosuvastatin lowers the serum cholesterol level most effectively, followed by atorvastatin, simvastatin and lovastatin. An additional hydrogen bond in rosuvastatin and atorvastatin (and the relatively hydrophilic property of the latter) offers additional bonding interactions with the catalytic site of HMG-CoA reductase (Liao 2002; Schachter, 2004).

In sensitive filamentous fungi, e.g. *A. nidulans*, treated with PAF, the main symptoms are inhibition of the spore germination and hyphal growth, retardation of the extension of the hyphae, induction of intracellular oxidative stress and an apoptosis-like phenotype (Marx, 2004). The influence of PAF in heterotrimeric G-protein signalling has also been proved. PAF evokes hyperpolarization of the plasma membrane of the sensitive mould *A. nidulans*. G-protein signalling plays an integral role, PAF most probably interacting directly or indirectly with the plasma membrane $H^+$ pump or triggering ion effluxes (Kaiserer *et al*., 2003; Leiter *et al*., 2005).

The activities of the statin-PAF combinations on the different strains varied and depended considerably on the activities of the components applied separately. When a strain was resistant to one of the components, e.g. *Rhizopus stolonifer* to PAF or *Mortierella wolfii* to the statins, significant interactions could not be detected; only the effect of the active component was observed. In comparison, Chamilos *et al*. (2006) demonstrated the synergistic activity of lovastatin with voriconazole (which alone has no appreciable activity against *Zygomycetes*) against different *Zygomycetes*. When the strains were to some extent sensitive to both types of antifungal agents, synergistic or additive effects were

detected, e.g. in the cases of *S. racemosum* and *Mycotypha africana*. An 80% decrease in the growth of *S. racemosum* was caused by $1\,\mu g\,mL^{-1}$ of rosuvastatin combined with $50\,\mu g\,mL^{-1}$ of PAF, and a $\geq 90\%$ decrease in the growth of *Mycotypha africana* was induced by the same concentrations of the combinations rosuvastatin-PAF and atorvastatin-PAF. In these fungi, complete growth inhibition was achieved with significantly lower statin concentrations when the statins were utilized together with PAF. It is important to mention that only one concentration of PAF was tested ($50\,\mu g\,mL^{-1}$); it is possible that inhibition can be induced with lower statin concentrations at higher concentrations of PAF.

It is presumed that statins and PAF are able to work together and generate a significant antifungal effect in sensitive *Zygomycetes*. It is important to take into account that administration of statins is contraindicated with azoles and other drugs that are metabolized by the cytochrome P450 in the liver (Herman, 1999; Chan *et al.*, 2003). No toxic effect of PAF on mammalian cells was earlier detected *in vitro* (Szappanos *et al.*, 2005). From this respect, investigation of the interactions between the two types of antifungal compounds may be fruitful for both basic and clinical investigations. The observed activities of statins, PAF (Marx, 2004) and statin-PAF combinations support the assumption that these compounds may be regarded as promising candidates in future antifungal drug research.

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