Activity of BAL 4815 against filamentous fungi

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Objectives: BAL 4815 is a new antifungal drug and it is the active component of the antifungal triazole BAL 8557 (the water-soluble prodrug). We studied the in vitro fungistatic and fungicidal activities of BAL 4815 against 103 clinical isolates of filamentous fungi, including 51 isolates of Aspergillus spp. and 52 isolates of non-Aspergillus filamentous fungi.

Methods: We evaluated the in vitro activity of BAL 4815 against 51 isolates of Aspergillus spp., 20 isolates of dematiaceous fungi, 18 isolates of hyaline Hyphomycetes and 14 isolates of Zygomycetes. MICs were determined following the CLSI M38-A broth microdilution method, using RPMI 1640 medium buffered to pH 7.0 with MOPS. Microdilution plates were incubated at 35°C and read at 24 and 48 h (Mucorales were read at 24 h). Minimal fungicidal concentrations were also determined.

Results: For all isolates, geometric mean MICs, MIC_{50}s, MIC_{90}s and MIC ranges (mg/L) were: Aspergillus spp., 1.67, 2, 4 and 0.5-4; dematiaceous fungi, 1.62, 1, >8 and 0.03 to >8; hyaline Hyphomycetes, 2.41, 2, >8 and 0.03 to >8; and Zygomycetes, 6.81, 8, >8 and 0.03 to >8. Differences in susceptibility between genera were noted. Scedosporium prolificans, Fusarium spp., Mucor spp. and Rhizopus spp. (MIC_{90} > 8 mg/L) were less susceptible than Aspergillus spp. (MIC_{90} = 4 mg/L).

Conclusions: BAL 4815 has excellent in vitro activity against Aspergillus spp. and variable activity against other filamentous fungi.

Keywords: Aspergillus, triazoles, antifungals

Introduction

Despite advances in antifungal therapy, invasive infections due to Aspergillus spp. and other filamentous fungi (moulds) have emerged as prominent causes of morbidity and mortality worldwide in immunocompromised hosts, remaining unacceptably high.1 Amphotericin B has been the key systemic antifungal therapy for many years; concerns regarding its toxicity have been partially addressed by the introduction of lipid formulations, but significant toxicity still remains, often causing therapy withdrawal.2 Since the discovery of the antifungal activity of the first azoles, huge advances have been made in this group to reduce toxicity, enhance bioavailability, improve the antifungal spectrum and counteract resistance. Voriconazole exhibits excellent in vitro and in vivo activity against Aspergillus species,3 and the new triazoles, ravuconazole and posaconazole have excellent in vitro activity against Aspergillus spp. and other filamentous fungi.4 BAL 4815 is a new, water-insoluble, investigational triazole with in vitro and in vivo activity against yeasts and moulds, and is the active antifungal component of BAL 8557 (the water-soluble prodrug suitable for oral and intravenous delivery);5 very low levels of cleavage product are detectable in the serum after oral or intravenous administration and it is not necessary to add potentially toxic cyclodextrin to increase or achieve solubility of this new azole as happens in itraconazole and voriconazole intravenous solutions. At the end of 1 h infusion of 50, 100 and 200 mg, C_{max} values of BAL 4815 reached 0.446, 1.03 and 2.47 mg/L, respectively. The corresponding AUC_{∞} values were 11.3, 26.6 and 73.2 μg·h/mL.6

We studied the in vitro activity of BAL 4815 against 103 isolates of filamentous fungi. Susceptibilities were determined using the broth microdilution method in accordance with the CLSI (formerly the NCCLS) reference method for microdilution antifungal susceptibility testing of filamentous fungi (M38-A).7

Materials and methods

Susceptibility tests were performed on 103 filamentous fungi isolates comprising 51 Aspergillus spp. (19 Aspergillus terreus,
14 Aspergillus fumigatus, 12 Aspergillus flavus, 3 Aspergillus glaucus, 2 Aspergillus niger and 1 Aspergillus nidulans), 20 dematiaceous fungi (7 Scedosporium apiospermum, 5 Scedosporium prolificans, 2 Rhinocladiella spp., 1 Hortaea werneckii, 1 Phialophora sp., 1 Stachybotrys sp., 1 Bipolaris sp. and 1 Phoma sp.), 18 hyaline Hyphomycetes (10 Fusarium spp., 2 Scopulariopsis spp., 2 Acremonium spp., 1 Trichoderma sp., 1 Verticillium sp., 1 Paecilomyces sp. and 1 Arthrobotrys sp.) and 14 Zygomycetes (6 Mucor spp., 5 Rhizopus spp., 1 Cunninghamella bertholletiae, 1 Ahsidia corymbifera and 1 Syncphalastrum sp.). These isolates were recovered from clinical specimens received in Valencia University Hospital in Seville (Spain), Puerta del Mar University Hospital in Cadiz (Spain) and La Fe University Hospital in Valencia (Spain). Identification of each isolate was performed using conventional mycological techniques. Mould isolates were maintained in sterile water and were subcultured on antimicrobial agent-free potato dextrose agar (Difco Laboratories, Detroit, MI, USA) to ensure viability and purity.

Candida krusei ATCC 6258, Candida parapsilosis ATCC 22019, A. flavus ATCC 204304 and A. fumigatus 204305 were included in each susceptibility test for quality control (QC) and assessment of reproducibility testing.

BAL 4815 (Basilea Pharmaceutica, Basel, Switzerland) was provided as a pure powder by the manufacturer and it was dissolved in 100% dimethyl sulphoxide. The broth microdilution test was done in accordance with the CLSI guidelines for filamentous fungi (CLSI document M38-A) using RPMI 1640 medium (Sigma Chemical Co., St Louis, MO, USA) buffered to pH 7.0 with MOPS (Sigma). Final drug range was 0.015–8 mg/L. Stock inoculum suspensions were prepared from 7-day-old cultures grown on potato dextrose agar following the CLSI guidelines (document M38-A). Stock suspensions were adjusted spectrophotometrically to optical densities that ranged from 0.09 to 0.11 (80% to 82% transmittance) and contained conidia or sporangiospores. The diluted (2-fold) inoculum sizes ranged from 0.9×10^4 to 4.7×10^5 cfu/mL, as demonstrated by quantitative colony counts on Sabouraud dextrose agar. Drug-free and cell-free controls were included. The microdilution plates were incubated and read after 24 and 48 h of incubation at 35°C except for the Zygomycetes, which were read at 24 h. The MIC endpoints were read visually with the aid of a reading mirror at the lowest drug concentration that prevented 100% growth at 24 h (Zygomycetes) and 48 h of incubation at 35°C.

The minimal fungicidal concentration (MFC) of each agent was determined according to Espinel-Ingroff et al. Briefly, 20 μL aliquots of broth were subcultured from each well that showed complete inhibition of growth (100%, or an optically clear well), from the growth control well (drug-free medium) and from the last positive well (with growth similar to that in the growth control well) onto Sabouraud dextrose agar (Oxoid, Basingstoke, UK) plates. The plates were incubated at 35°C until growth was seen in the growth control subculture (usually 48 h later). The MFC was defined as the lowest drug concentration that resulted in either no growth or growth of fewer than three colonies, which corresponds to a killing activity of 99.0% to 99.5%. The drug showed fungicidal activity when MFC was within two dilutions of the MIC.

For analysis of the results, geometric means (GMs), ranges of MICs, MIC50 and MIC90 values were calculated. MIC50 and MIC90 values were calculated as the lowest drug concentrations that prevented 100% growth of 50% and 90% of all the isolates, respectively, and MFC50 and MFC90 values were calculated as the lowest drug concentrations that prevented 99.0% to 99.5% growth of 50% and 90% of the isolates, respectively.

### Results

Table 1 shows the results of the in vitro susceptibility values against 103 filamentous fungi obtained after 48 h of incubation except for Zygomycetes, which could not be interpreted at 48 h, as they had no MIC by over growth. The GM MICs, MIC ranges and MIC50/MIC90 values (mg/L) were, respectively: Aspergillus spp., 1.67, 0.5–4 and 2/4; dematiaceous fungi, 1.62, 0.03 to >8 and 1/8; hyaline Hyphomycetes, 2.41, 0.03 to >8 and 2/8; and Zygomycetes, 6.81, 0.5 to >8 and 8/8.

For Aspergillus spp., BAL 4815 showed higher activity against A. terreus (MIC50/MIC90 = 1/2 mg/L) than the other species of Aspergillus (MIC50/MIC90 = 2/4 mg/L).

For non-Aspergillus filamentous fungi, the activity of BAL 4815 was variable. For dematiaceous fungi, BAL 4815 showed higher activity against non-Scedosporium spp. isolates (MIC50 and MIC90 of 0.5 and 4 mg/L, respectively) than for Scedosporium spp. (MIC50 = 1 and MIC90 = ≥8 mg/L). For hyaline Hyphomycetes, BAL 4815 was more active against isolates of Scopulariopsis spp., Trichoderma spp., Verticillium spp., Paecilomyces spp. and Arthrobotrys spp. (MIC50 = 0.5 and MIC90 = 4 mg/L) than for Fusarium spp. (MIC50 = 4 and MIC90 = >8 mg/L) and Acremonium spp. (MIC = >8 mg/L). For Zygomycetes, MIC50/MIC90 values were high for Mucor spp. and Rhizopus spp. (8 and >8 mg/L, respectively), and in other Zygomycetes, the activity of BAL 4815 was variable: As. corymbifera, MIC = 1; Syncphalastrum, MIC = 4; and C. bertholletiae, MIC = >8 mg/L.

For all isolates, GM MFC values and ranges (mg/L) were: Aspergillus spp., 3.77 and 1–8; dematiaceous fungi, 0.99 and 0.03 to >8; hyaline Hyphomycetes, 1.49 and 0.06 to >8; and Zygomycetes, 4 and 1 to >8. BAL 4815 was fungicidal in 98.5%, and the MFC50 and MFC90 were, respectively: Aspergillus spp., 3.77 and 1–8; dematiaceous fungi, 0.99 and 0.03 to >8; and Zygomycetes, 4.0 and 8.0.

MFCs were determined only for wells that showed complete inhibition of growth. We calculated GM MFCs and MFC50/90s only when 10 or more strains were available.

MIC50/90s and MFCs (mg/L) of QC strains were: C. krusei ATCC 6258, 0.5/1 and 4; C. parapsilosis ATCC 22019, 0.5/1 and 2; A. flavus ATCC 204304, 2/4 and 8; and A. fumigatus 204305, 1/2 and 2.

The MFC value was the same as or only one dilution higher than the MIC for 92% of Aspergillus spp., 100% of dematiaceous fungi, 92% of hyaline Hyphomycetes and 100% of Zygomycetes (the isolates with an MIC of 8 mg/L of BAL 4815 were not used in this analysis of the data). The reproducibility study on 19% of the isolates showed that 75% of isolates retested were within one dilution of the original MIC value of BAL 4815.

### Discussion

There are few studies that have evaluated the activity of this new antifungal azole against filamentous fungi and they include a low number of isolates. Our results are difficult to compare with those of other authors who also tested filamentous fungi because they used other azoles.
Recently, Warn et al.\(^9\) reported that BAL 4815 showed primary fungicidal activity against all four Aspergillus species tested (A. fumigatus, A. terreus, A. flavus and A. niger), finding that A. terreus was more susceptible to BAL 4815 than A. flavus and A. niger, which is in agreement with the results obtained in our study.

In relation to non-Aspergillus filamentous fungi, MIC\(_{50}\) values for dematiaceous fungi, hyaline Hyphomycetes and Zygomycetes in our study were lower than those obtained by Gonzalez and Heep.\(^{10}\) Non-Scedosporium dematiaceous fungi and other hyaline Hyphomycetes (Paecilomyces sp.) had an MIC\(_{50}\) of 0.5 mg/L. Fusarium spp. had an MIC\(_{50}\) of 4 mg/L and A. corymbifera had an MIC\(_{50}\) of 1 mg/L. Gonzalez and Heep obtained an MIC\(_{50}\) of 2 mg/L for non-Scedosporium dematiaceous fungi, an MIC\(_{50}\) of 1 mg/L for other hyaline Hyphomycetes, an MIC\(_{50}\) of 8 mg/L for Fusarium spp. and an MIC\(_{50}\) of 4 g/L for A. corymbifera. Mucor spp. and Rhizopus spp. had the same values or slightly higher in our study, and the MIC ranges, MIC\(_{90}\)/MIC\(_{90}\) and GM at 48 h were slightly higher than those presented by Warn et al.\(^{11}\)

In the present study, the non-Aspergillus filamentous fungi most susceptible to BAL 4815 were the non-Scedosporium dematiaceous fungi (Rhniocladiella spp., H. werneckii, Phialophora sp., Stachybotrys sp., Curvularia sp., Bipolaris sp. and Phoma sp.). BAL 4815 did not show fungicidal activity against S. prolificans (MIC\(_{90}\) > 8 mg/L); there are no published data with BAL 4815 and this species, although other authors also found MICs/MFCs > 8 mg/L of posaconazole, itraconazole and voriconazole for S. prolificans.\(^{12,13}\)

The new triazoles (posaconazole, ravuconazole and posaconazole) have demonstrated some activity against miscellaneous moulds, but none of these agents had good in vitro activity against Fusarium spp. (MIC\(_{90}\) > 8 mg/L).\(^{12-16}\) Our findings for BAL 4815 against Fusarium spp. are in agreement with previously published in vitro data for other azoles.\(^{12,13}\)

We report in vitro results in agreement with other authors\(^{12-16}\) for 14 Zygomycetes, although the Zygomycetes are a heterogeneous group with regard to antifungal susceptibility testing.\(^{17}\) Warn et al.\(^9\) demonstrated that BAL 4815 showed primary fungicidal activity (MFC within two dilutions of the MIC) against all Aspergillus species tested. In our study against Aspergillus spp. and non-Aspergillus spp., 98.7% of isolates had the MFC within one dilution of the MIC.

Indeed, 71% of isolates of Aspergillus spp. were killed at \(<\)2.0 mg/L BAL 4815, in agreement with Warn et al.\(^5\) and 25% of isolates of non-Aspergillus filamentous fungi were killed at 4 mg/L.

In summary, we found that BAL 4815 has excellent in vitro activity against Aspergillus spp. and variable activity against other filamentous fungi. Therefore, further in vitro and in vivo studies are necessary in order to verify the antifungal activity of this azole.

Table 1. In vitro susceptibilities of 103 isolates of filamentous fungi to BAL 4815

<table>
<thead>
<tr>
<th>Species (no. of isolates)</th>
<th>GM(^a) MIC/MFC(^b) (mg/L)</th>
<th>Range MIC/MFC(^b) (mg/L)</th>
<th>MIC(<em>{50})/MIC(</em>{90}) (mg/L)</th>
<th>MIC(<em>{50})/MIC(</em>{90}) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus spp. (51)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. terreus (19)</td>
<td>0.96/2.31</td>
<td>0.5–4/1–8</td>
<td>1/2</td>
<td>2/8</td>
</tr>
<tr>
<td>A. fumigatus (14)</td>
<td>1.90/4.64</td>
<td>1–4/2–8</td>
<td>2/4</td>
<td>2/8</td>
</tr>
<tr>
<td>A. flavus (12)</td>
<td>2.41/4.75</td>
<td>1–4/2–8</td>
<td>2/4</td>
<td>4/8</td>
</tr>
<tr>
<td>A. glaucus (3)</td>
<td>4/ND</td>
<td>4/ND</td>
<td>ND/ND</td>
<td>ND/ND</td>
</tr>
<tr>
<td>A. niger (2)</td>
<td>2.82/ND</td>
<td>2–4/ND</td>
<td>ND/ND</td>
<td>ND/ND</td>
</tr>
<tr>
<td>A. nidulans (1)</td>
<td>4/ND</td>
<td>4/ND</td>
<td>ND/ND</td>
<td>ND/ND</td>
</tr>
<tr>
<td>total</td>
<td>1.67/3.77</td>
<td>0.5–4/1–8</td>
<td>2/4</td>
<td>4/8</td>
</tr>
<tr>
<td>Dematiaceous fungi (20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. prolificans (5)</td>
<td>4.59/ND</td>
<td>1 to &gt;8/8</td>
<td>1/ND</td>
<td>&gt;8/ND</td>
</tr>
<tr>
<td>S. apiospermum (7)</td>
<td>2.97/ND</td>
<td>0.25 to &gt;8/ND</td>
<td>1/ND</td>
<td>8/ND</td>
</tr>
<tr>
<td>other dematiaceous fungi (8)(^d)</td>
<td>0.49/ND</td>
<td>0.03–4/ND</td>
<td>0.5/ND</td>
<td>4/ND</td>
</tr>
<tr>
<td>total</td>
<td>1.62/0.99</td>
<td>0.03 to &gt;8/0.03 to &gt;8</td>
<td>1/1</td>
<td>&gt;8/8</td>
</tr>
<tr>
<td>Hyaline fungi (18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium spp. (10)</td>
<td>4/2.82</td>
<td>0.25 to &gt;8/1–8</td>
<td>4/4</td>
<td>&gt;8/8</td>
</tr>
<tr>
<td>other hyaline</td>
<td>1.29/ND</td>
<td>0.03 to &gt;8/ND</td>
<td>2/ND</td>
<td>&gt;8/ND</td>
</tr>
<tr>
<td>Hyphomycetes (8)(^e)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>total</td>
<td>2.41/1.49</td>
<td>0.03 to &gt;8/0.06 to &gt;8</td>
<td>2/2</td>
<td>&gt;8/8</td>
</tr>
<tr>
<td>Zygomycetes (14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizopus spp. (5)</td>
<td>9.18/ND</td>
<td>4 to &gt;8/ND</td>
<td>8/ND</td>
<td>&gt;8/ND</td>
</tr>
<tr>
<td>Mucor spp. (6)</td>
<td>6.96/ND</td>
<td>0.5 to &gt;8/ND</td>
<td>8/ND</td>
<td>&gt;8/ND</td>
</tr>
<tr>
<td>other Zygomycetes (3)(^f)</td>
<td>4/ND</td>
<td>1 to &gt;8/ND</td>
<td>1/ND</td>
<td>4/ND</td>
</tr>
<tr>
<td>total</td>
<td>6.81/4</td>
<td>0.5 to &gt;8/1 to &gt;8</td>
<td>8/4</td>
<td>&gt;8/8</td>
</tr>
<tr>
<td>Total 103</td>
<td>2.13/2.60</td>
<td>0.03 to &gt;8/0.06 to &gt;8</td>
<td>2/4</td>
<td>8/8</td>
</tr>
</tbody>
</table>

\(^a\) In calculation of the GM values, MICs > 8 mg/L were classed as 16 mg/L.

\(^b\) MFCs were only determined for wells that showed complete inhibition of growth.

\(^c\) 50% and 90%, MICs at which 50% and 90% of isolates were inhibited, respectively.

\(^d\) Includes: Phialophora sp., Stachybotrys sp., Curvularia sp., Bipolaris sp., Phoma sp., Rhinocladia sp. and Horta sp. werneckii.

\(^e\) Includes: Scopulariopsis sp., Acremonium sp., Trichoderma sp., Verticillium sp., Paecilomyces sp. and Arthrographis sp.

\(^f\) Includes: A. corymbifera, Syncephalastrum sp. and C. bertholletiae.
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Transparency declarations

None to declare.

References