Activity of BAL 4815 against filamentous fungi

C. Martín de la Escalera1*, A. I. Aller1, E. López-Oviedo1, A. Romero1, A. I. Martos1, E. Cantón2, J. Pemán2, P. García Martos3 and E. Martín-Mazuelos1

1Servicio de Microbiología y Parasitología, Hospital Universitario de Valme, Carretera de Cádiz s/n, Sevilla, Spain; 2Hospital La Fe, Avda. Campanar 21, Valencia 46009, Spain; 3Hospital Puerta del Mar, Avda. Ana de Viya 21, Cádiz, 11009, Spain

Received 14 May 2007; returned 3 July 2007; revised 28 January 2008; accepted 1 February 2008

Objectives: BAL 4815 is a new antifungal drug and it is the active component of the antifungal triazole BAL 8557 (the water-soluble precursor). 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, MIC50s, MIC90s 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. (MIC90 > 8 mg/L) were less susceptible than Aspergillus spp. (MIC90 = 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 newazole as happens in itraconazole and voriconazole intravenous solutions. At the end of 1 h infusion of 50, 100 and 200 mg, Cmax 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, 20 Aspergillus fumigatus, 5 Aspergillus niger, 4 Aspergillus nidulans, 1 Aspergillus flavus, 1 Aspergillus Brasiliensis, 4 Aspergillus flavus, 1 Aspergillus niger, 1 Aspergillus fumigatus and 1 Aspergillus terreus), 20 isolates of dematiaceous fungi (14 species), 18 isolates of hyaline Hyphomycetes and 14 isolates of Zygomycetes. Susceptibilities 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

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 = 50.5 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: A. corymbifera, MIC = 1; Syncephalastrum, 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., 4.0 and 8.0; dematiaceous fungi, 1.0 and 8.0; hyaline Hyphomycetes, 2.0 and 8.0; and Zygomycetes, 4.0 and 8.0.

MFCs were determined only for wells that showed complete inhibition of growth. We calculated GM MFCs and MFC90/90s only when 10 or more strains were available.

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., 4.0 and 8.0; dematiaceous fungi, 1.0 and 8.0; hyaline Hyphomycetes, 2.0 and 8.0; and Zygomycetes, 4.0 and 8.0.

MFCs were determined only for wells that showed complete inhibition of growth. We calculated GM MFCs and MFC90/90s only when 10 or more strains were available.

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., 4.0 and 8.0; dematiaceous fungi, 1.0 and 8.0; hyaline Hyphomycetes, 2.0 and 8.0; and Zygomycetes, 4.0 and 8.0.

MFCs were determined only for wells that showed complete inhibition of growth. We calculated GM MFCs and MFC90/90s only when 10 or more strains were available.

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., 4.0 and 8.0; dematiaceous fungi, 1.0 and 8.0; hyaline Hyphomycetes, 2.0 and 8.0; and Zygomycetes, 4.0 and 8.0.

MFCs were determined only for wells that showed complete inhibition of growth. We calculated GM MFCs and MFC90/90s only when 10 or more strains were available.

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., 4.0 and 8.0; dematiaceous fungi, 1.0 and 8.0; hyaline Hyphomycetes, 2.0 and 8.0; and Zygomycetes, 4.0 and 8.0.

MFCs were determined only for wells that showed complete inhibition of growth. We calculated GM MFCs and MFC90/90s only when 10 or more strains were available.

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., 4.0 and 8.0; dematiaceous fungi, 1.0 and 8.0; hyaline Hyphomycetes, 2.0 and 8.0; and Zygomycetes, 4.0 and 8.0.

MFCs were determined only for wells that showed complete inhibition of growth. We calculated GM MFCs and MFC90/90s only when 10 or more strains were available.
### Activity of BAL 4815 against filamentous fungi

#### 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})/MFC(</em>{50}) (mg/L)</th>
<th>MIC(<em>{90})/MFC(</em>{90}) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em> spp. (51)</td>
<td>A. terreus (19) 0.96/2.31</td>
<td>0.5–4/1–8</td>
<td>1/2</td>
<td>2/8</td>
</tr>
<tr>
<td></td>
<td>A. fumigatus (14) 1.90/4.64</td>
<td>1–4/2–8</td>
<td>2/4</td>
<td>2/8</td>
</tr>
<tr>
<td></td>
<td>A. flavus (12) 2.41/4.75</td>
<td>1–4/2–8</td>
<td>2/4</td>
<td>4/8</td>
</tr>
<tr>
<td></td>
<td>A. glaucus (3) 4/ND</td>
<td>4/ND</td>
<td>ND/ND</td>
<td>ND/ND</td>
</tr>
<tr>
<td></td>
<td>A. niger (2) 2.82/ND</td>
<td>2–4/ND</td>
<td>ND/ND</td>
<td>ND/ND</td>
</tr>
<tr>
<td></td>
<td>A. nidulans (1) 4/ND</td>
<td>4/ND</td>
<td>ND/ND</td>
<td>ND/ND</td>
</tr>
<tr>
<td></td>
<td>total 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>S. prolificans (5) 4.59/ND</td>
<td>1 to &gt;8/ND</td>
<td>1/ND</td>
<td>&gt;8/ND</td>
</tr>
<tr>
<td></td>
<td>S. apiospermum (7) 2.97/ND</td>
<td>0.25 to &gt;8/ND</td>
<td>1/ND</td>
<td>8/ND</td>
</tr>
<tr>
<td></td>
<td>other dematiaceous fungi (8)(^c) 0.49/ND</td>
<td>0.03–4/ND</td>
<td>0.5/ND</td>
<td>4/ND</td>
</tr>
<tr>
<td></td>
<td>total 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 Hyphomycetes (18)</td>
<td>Fusarium spp. (10) 4.2/82</td>
<td>0.25 to &gt;8/1&gt;8</td>
<td>4/4</td>
<td>&gt;8/8</td>
</tr>
<tr>
<td></td>
<td>other hyaline 1.29/ND</td>
<td>0.03 to &gt;8/ND</td>
<td>2/ND</td>
<td>&gt;8/ND</td>
</tr>
<tr>
<td></td>
<td>Hyphomycetes (8)(^d) total 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>Zygomyces (14)</td>
<td>Rhizopus spp. (5) 9.18/ND</td>
<td>4 to &gt;8/ND</td>
<td>8/ND</td>
<td>&gt;8/ND</td>
</tr>
<tr>
<td></td>
<td>Mucor spp. (6) 6.96/ND</td>
<td>0.5 to &gt;8/ND</td>
<td>8/ND</td>
<td>&gt;8/ND</td>
</tr>
<tr>
<td></td>
<td>other Zygomyces (3)(^f) total 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>

ND, not determined.

\(^a\)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., *Rhinocladiella* spp. and *Hortaea 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*.

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 Zygomyces 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\(_{90}\)/MFC\(_{90}\) and GM MICs 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 (*Rhinocladiella* 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\(_{50}\) > 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 Zygomyces, although the Zygomyces 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.\(^9\) 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.
de la Escalera et al.

Acknowledgements
We thank Basilea Pharmaceutica for providing BAL 4815 as a pure powder at no cost.

Funding
This work was supported in part by the Unit of Investigation of Microbiology of Valme University Hospital. No other funding was provided for the work.

Transparency declarations
None to declare.

References

European Society of Clinical Microbiology and Infectious Diseases, Basel, Switzerland.