P2020

University Hospitals Cleveland Medical Center

Case Western Reserve University School of Medicine

Evaluation of the Effect of Ibrexafungerp Alone and in Combination with Amphotericin B or Posaconazole against *Mucor* Strains on Time Kill Kinetics and Scanning Electron Microscopy

Mahmoud Ghannoum, Thomas McCormick, Lisa Long, Rachael Gowen

Abstract

Invasive molds opportunistically infect vulnerable populations such as immunocompromised patients, especially in nosocomial settings. The orders *Aspergillus* and *Mucorales* are thought to constitute the majority of these pathogenic infections. Environmental molds can then become life-threatening, and affected individuals are at the mercy of current antifungals. However, antifungal resistance of mold species in combination with other limiting factors, such as drug-drug interactions and toxicity prevent full effectiveness of treatment. Consequently, novel treatments are necessary to combat drug resistance. One such antifungal is Ibrexafungerp, a glucan synthase inhibitor, which is the first in a new class of triterpenoid antifungals. The Ibrexafungerp mechanism of action is similar to echinocandin antifungals, but does not currently exhibit any resistance among pathogenic fungi and molds. Ibrexafungerp to date demonstrates effective treatment against *Aspergillus* pathogens, but further orders such as *Mucorales* have yet to be entirely investigated. Therefore, Ibrexafungerp is a promising alternative for treating antifungal-resistant molds and offers newfound protection for vulnerable and affected populations.

Introduction

- Invasive molds opportunistically infect vulnerable populations
- *Aspergillus* and *Mucorales* are thought to constitute the majority of these pathogenic infections
- Antifungal resistance of mold species in combination with other limiting factors prevent full effectiveness of treatment.
- Novel treatments are necessary to combat drug resistance
- Ibrexafungerp, a glucan synthase inhibitor, is the first in a new class of triterpenoid antifungals
- IBX in combination with other antifungals is a promising alternative for treating antifungal-resistant molds and offers newfound protection for vulnerable and affected populations.
- **In this study we assessed the activity of IBX alone and in combination with amphotericin B or Posaconazole against *Mucor* strains.**

Materials & Methods

Time Kill Evaluation

This time-kill analysis was conducted according to the methods described by Klepser et. al. Briefly, suspensions of test organisms were added to 100 mL of YNB broth containing concentrations of IBX at 2× MIC or comparator each at 0.25 and 0.5× MIC for each isolate, alone or in combination (see Table 1). At predetermined time points (0, 1, 4, 6, 24, and 48 hours), a 0.1 mL sample was removed and diluted with normal saline (0.85%). A 30 µL aliquot from each dilution was then plated onto a potato dextrose agar plate. Colony forming units (CFUs) were determined following 2-3 days incubation. Each experiment was carried out in duplicate, and a time-kill curve was plotted for each isolate.

Scanning Electron Microscopy (SEM)

The effect of IBX alone and in combination with AmB on the morphology and ultrastructure of the Mucor strains was determined using SEM as described previously by Chandra et al. Briefly, *Mucor* strains were exposed overnight to IBX or AmB, or combination of both at 35 °C. Next, 200 µL of cell suspension was fixed in 2% glutaraldehyde and incubated at 4 °C for 48 h. After fixation, samples were processed and dried.

Materials & Methods Cont.

Processed samples were coated with palladium for 60 s and viewed with the Nova NanoLab 200 FEG-SEM/FIB scanning electron microscope in high-vacuum mode at 2.00 kV. Untreated cells were included as controls for each strain. Images captured for each set of samples were analyzed for morphological and ultrastructural changes.

Results: Scanning Electron Microscopy

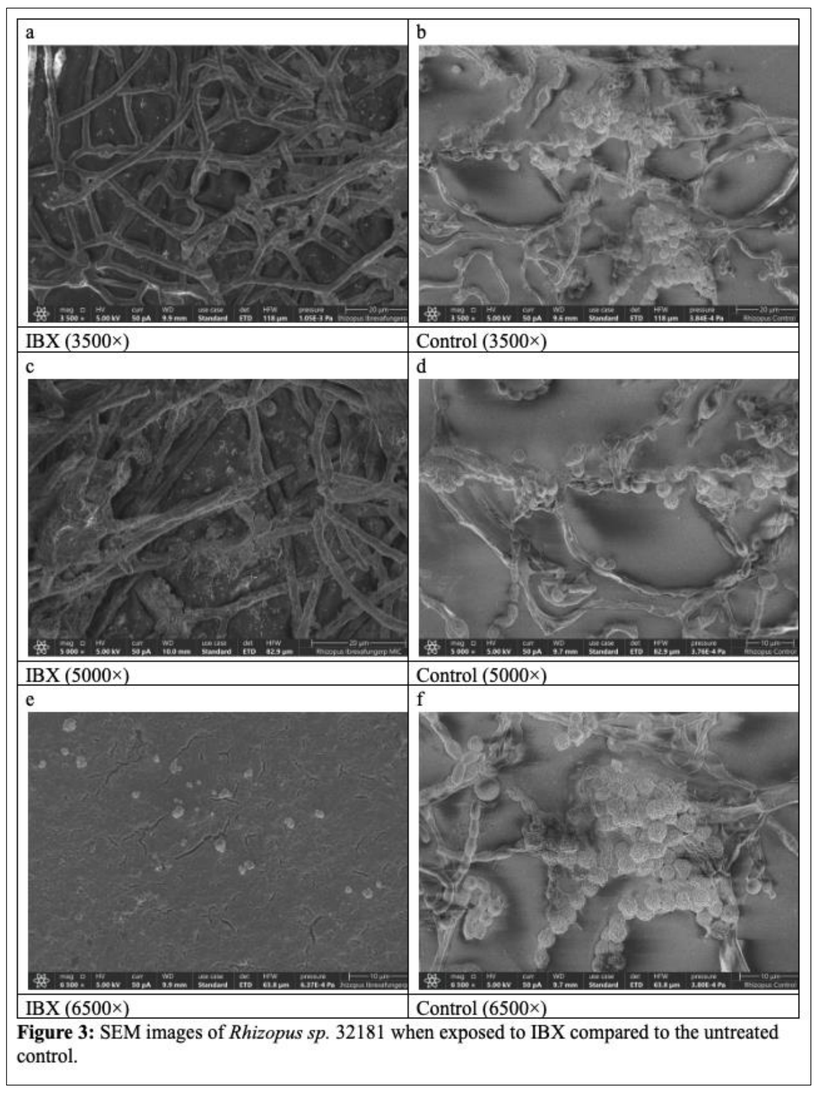

Figure 3: SEM images of *Rhizopus sp.* 32181 when exposed to IBX compared to the untreated control.

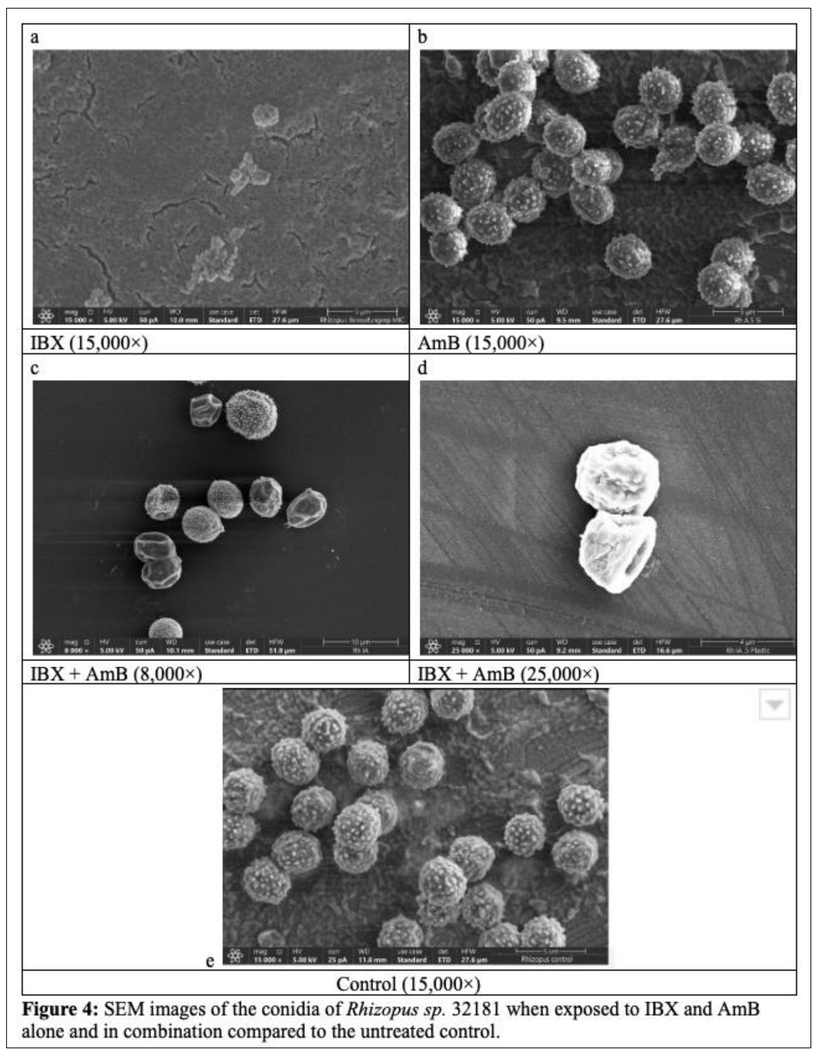

Figure 4: SEM images of the conidia of *Rhizopus sp.* 32181 when exposed to IBX and AmB alone and in combination compared to the untreated control.

Species	MRL	IBX		AmB		FICI	Interpretation
		Alone	Combination	Alone	Combination		
Rhizopus sp.	32181	32	2	2	0.5	0.3	Synergistic
Cunninghamella sp.	1176	32	0.5	4	2	0.5	Synergistic
Rhizomucor oryzae	43156	16	0.5	1	0.5	0.5	Synergistic
Species	**MRL**	**IBX**		**POS**		**FICI**	**Interpretation**
		Alone	**Combination**	**Alone**	**Combination**		
Rhizopus sp.	32181	32	0.03	4	0.03	0.0	Synergistic
Cunninghamella sp.	1176	32	8	2	0.25	0.4	Synergistic
Rhizomucor oryzae	43156	16	1	0.5	0.5	1.1	No interactions

Table 1: Selected isolates tested to evaluate the effect of IBX alone and in combination against *Mucor* isolates (µL/mL).

Results: Time Kill Kinetics

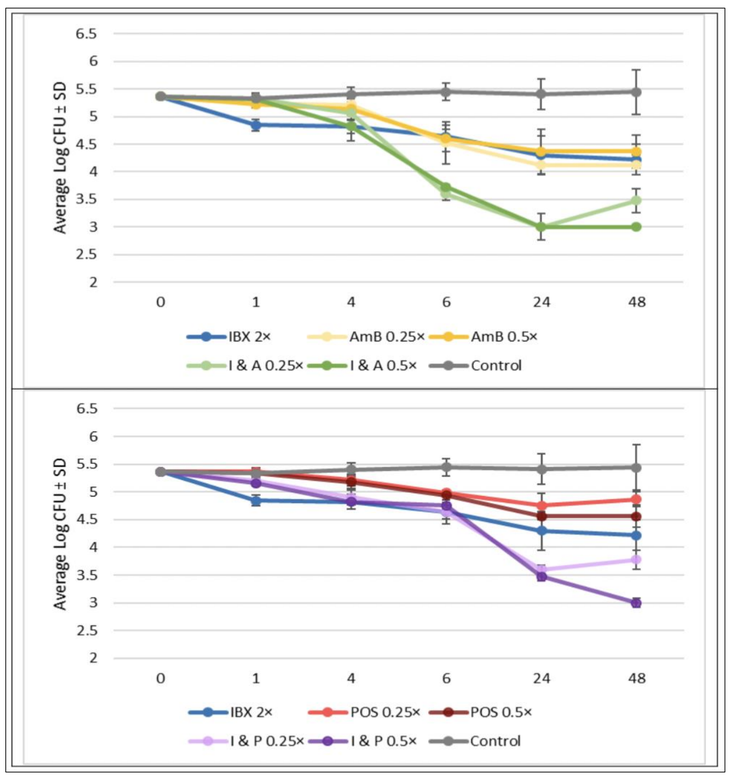

Figure 1: Average log CFUs/mL of *Rhizopus* sp. 32181 when exposed to IBX (2× MIC) alone and in combination with a) AmB (0.25 and 0.5× MIC) and b) POS (0.25 and 0.5× MIC) are shown.

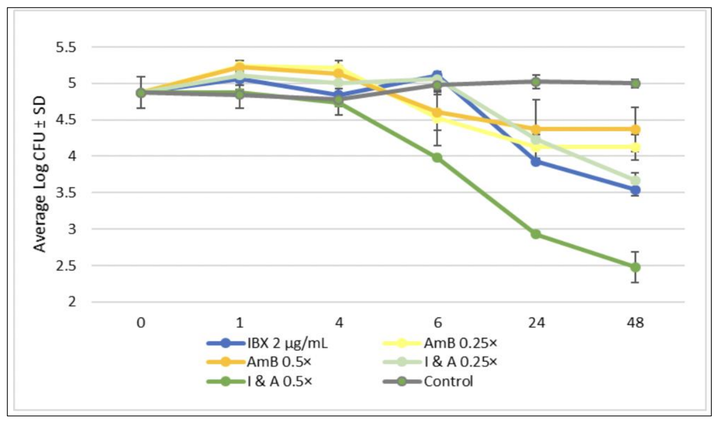

Figure 2: Time kill kinetics of IBX and Amphotericin B alone or in combination. Average log CFUs/mL of *Cunnighamella* sp. 1176 when exposed to IBX 2 µg/mL alone and in combination with AmB (0.25 and 0.5× MIC) are shown.

Conclusion

Ibrexafungerp in combination or alone is effective at treating various fungal infections including: *Cunninghamella, Rhizopus oryzae, Rhizopus sp,* and potentially other *Mucor* species. Ibrexafungerp alone demonstrated an average log decrease of 1-1.5 when CFU was calculated. Additionally, Ibrexafungerp induced morphological changes that inhibited cell growth or induced apoptosis. IBX in combination with Amphotericin B generally saw a 2-fold log decrease in CFU, with IBX in combination with Posaconazole achieving similar fold decreases. SEM images reveal clear morphological differences: IBX alone exbibits cell death and prevention of conidial growth. In combination with Amphotericin B, IBX caused widespread apoptosis and very limited cell density.

Acknowledgements This work was supported by Scynexis, Inc.

33rd ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Copenhagen, Denmark
15–18 April 2023