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Evaluation of the Post-Antifungal Effect of Rezafungin and Micafungin against *Candida albicans, Candida parapsilosis,* and *Candida glabrata*

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Introduction

- Post-antifungal effect (PAFE) is defined as the growth suppression of fungal cells after their exposure to an antifungal agent.
- Understanding PAFE helps evaluate dosage regimens for an antifungal agent.
- Rezafungin is a new echinocandin with an extended half-life that exhibits activity against *Candida* spp., *Aspergillus* spp., and *Pneumocystis* spp. and is in Phase 3 clinical development.
- In this study, the PAFE of rezafungin was compared to that of micafungin against *Candida albicans*, *C. glabrata*, and *C. parapsilosis* isolates.

Materials and Methods

- Six *Candida* spp. isolates were tested, including 2 *C. albicans* (ATCC 90028 and #1 clinical isolate), 2 *C. parapsilosis* (ATCC 22019 and #2 clinical isolate), and 2 *C. glabrata* (#3 and #4 clinical isolates).
- Antifungal susceptibility testing was performed using the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution method.
  - Rezafungin (Cidara Therapeutics) and micafungin (Sigma-Aldrich) were tested.
  - Each isolate was tested in triplicate to establish baseline MIC values (bMIC).
  - Modal MIC values were used to determine drug concentrations for PAFE testing.
- For PAFE determinations, antifungal concentrations of 1X, 4X, and 16X the baseline MIC were used.
- A starting inoculum of 1-5x10^5 CFU/mL from a fresh culture was added to RPMI with the respective antifungal at the desired concentration (1X, 4X, or 16X the bMIC).
  - A growth control that was not exposed to an antifungal agent was used to determine the standard 1-$\log_{10}$ increase.
- After a 1-h exposure to the antifungal agent, the cells were washed three times with RPMI and reconstituted with pre-warmed RPMI to a final volume of 10mL.
- Colony counts were performed at T0 (pre-exposure), after the 1-h drug exposure, and after the cell wash (T1).
  - Test cultures were re-incubated following final cell wash.
  - Colony counts were performed at T2, T4, T8, T12, T24, and T48 hours.
- PAFE was calculated as the difference in time required for isolates to grow 1-$\log_{10}$ after the final cell wash compared to the untreated growth control.
  - PAFE = T – C, where T is time required for isolate to increase 1-$\log_{10}$ (in CFU) after drug removal and C is time required for untreated growth control to increase 1-$\log_{10}$ after undergoing the same process performed on the test culture.
- The reduction in starting inocula, in log-kill, was also calculated over the 48-h study period.

Results

- PAFE and bMIC results for rezafungin and micafungin are shown in Table 1.
- *C. albicans*
  - Rezafungin and micafungin PAFEs were >14.9 h against the *C. albicans* clinical isolate for all concentrations tested.
  - The *C. albicans* ATCC 90028 control failed to re-grow over 1-$\log_{10}$; the rezafungin and micafungin PAFEs could not be determined against this isolate.
- *C. glabrata*
  - Rezafungin PAFE results were >40 h for both *C. glabrata* strains, regardless of the concentration tested.
  - Micafungin PAFE results were equivalent to rezafungin PAFE values (>40 h) for *C. glabrata* isolates at all concentrations, except the 1X bMIC for the *C. glabrata* #3 isolate.
  - The micafungin PAFE value was 20.4 h for *C. glabrata* #3 isolate, while the rezafungin PAFE was >46.7 h.
- *C. parapsilosis*
  - Rezafungin PAFE results were also >40 h against the *C. parapsilosis* ATCC 22019 strain at all concentrations.
  - In contrast, no micafungin PAFE was observed against *C. parapsilosis* ATCC 22019 at 1X and 4X bMIC, and a short PAFE (1.8 h) was noted at 16X bMIC.
  - The *C. parapsilosis* clinical isolate #2 displayed prolonged rezafungin PAFE values (range, 18.4 h to >36.6 h), regardless of the concentration tested.
  - A short PAFE was displayed by micafungin at 1X (1.6 h) and 4X (7.4 h) bMIC against *C. parapsilosis* #3 while a PAFE of 31.3 h was noted for micafungin against this clinical isolate at 16X bMIC.

Conclusion

- Rezafungin showed sustained growth inhibition following drug removal and displayed equivalent or longer PAFE values than micafungin against all tested *Candida* spp.

Acknowledgements

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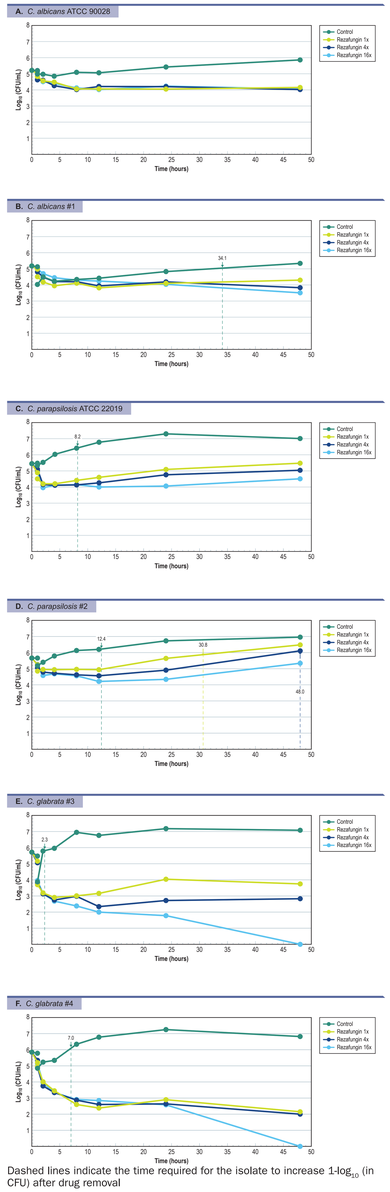
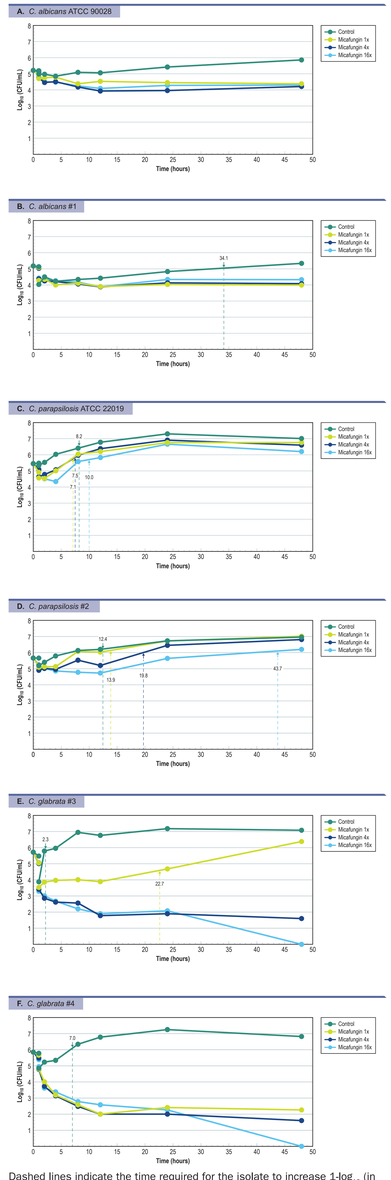
Figure 1. Rezafungin PAFE against *C. albicans*, *C. parapsilosis*, and *C. glabrata* at 1X, 4X, and 16X the baseline MIC values



Dashed lines indicate the time required for the isolate to increase 1-$\log_{10}$ (in CFU) after drug removal

Figure 2. Micafungin PAFE against *C. albicans*, *C. glabrata*, and *C. parapsilosis* at 1X, 4X, and 16X the baseline MIC values



Dashed lines indicate the time required for the isolate to increase 1-$\log_{10}$ (in CFU) after drug removal

Table 1. PAFE for rezafungin and micafungin against *C. albicans*, *C. parapsilosis*, and *C. glabrata*

Antifungal/Strain	Baseline MIC (mg/L)	PAFE (hours) at the following multiple of baseline MIC		
		1X	4X	16X
Rezafungin				
C. albicans ATCC 90028	0.03	ND	ND	ND
C. albicans #1	0.06	>14.9	>14.9	>14.9
C. parapsilosis ATCC 22019	1	>40.8	>40.8	>40.8
C. parapsilosis #2	1	18.4	35.6	>36.6
C. glabrata #3	0.12	>46.7	>46.7	>46.7
C. glabrata #4	0.12	>42.0	>42.0	>42.0

Antifungal/Strain	Baseline MIC (mg/L)	PAFE (hours) at the following multiple of baseline MIC		
		1X	4X	16X
Micafungin				
C. albicans ATCC 90028	0.03	ND	ND	ND
C. albicans #1	0.015	>14.9	>14.9	>14.9
C. parapsilosis ATCC 22019	1	≤0.0	≤0.0	1.8
C. parapsilosis #2	1	1.6	7.4	31.3
C. glabrata #3	0.06	20.4	>46.7	>46.7
C. glabrata #4	0.03	>42.0	>42.0	>42.0

Abbreviations: ND, not determined