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SUSCEPTIBILITY

Rifampin Enhances the Activity of Amphotericin B against *Fusarium solani* Species Complex and *Aspergillus flavus* Species Complex Isolates from Keratitis Patients

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ABSTRACT The *in vitro* activities of amphotericin B in combination with rifampin were assessed against 95 ocular fungal isolates. The interactions between amphotericin B and rifampin at 4, 8, 16, and 32 μg/ml were synergistic for 11.8%, 51.0%, 90.2%, and 94.1%, respectively, of *Fusarium solani* species complex isolates and for 13.6%, 45.5%, 93.2%, and 95.5%, respectively, of *Aspergillus flavus* species complex isolates. Antagonism was never observed for the amphotericin B-rifampin combinations.

KEYWORDS amphotericin B, *Aspergillus flavus* species complex, *Fusarium solani* species complex, rifampin, synergistic activity, fungal keratitis

Keratomycosis is a major cause of vision loss in developing countries like China because of higher incidence and the unavailability of effective antifungal agents (1–3). The *Fusarium solani* species complex (FSSC) and *Aspergillus flavus* species complex (AFSC) are two predominant ocular fungal pathogens and are thought to be particularly virulent, more resistant to antifungals, and have worse outcomes than other species of *Fusarium* and *Aspergillus* in China and in many other parts of the world (4–10). Keratomycosis is notoriously difficult to treat. Amphotericin B is one of the most commonly used topical agent to treat keratomycosis (11, 12); however, nonsusceptibility to amphotericin B has been recently reported for filamentous fungi (13–16), and some studies have shown that the response rates to amphotericin B for *Fusarium* keratitis and *Aspergillus* keratitis are 56% and 27%, respectively (11, 17, 18). Therefore, there is an urgent need for new approaches to manage amphotericin B-nonsusceptible filamentous fungi. One possible approach is to combine amphotericin B with other antimicrobial agents (12). Amphotericin B and natamycin are often combined in the treatment of keratomycosis. However, a study by Lalitha et al. has shown that amphotericin B and natamycin are not synergistic *in vitro* against *Fusarium* and *Aspergillus* species isolated from keratitis (19). Two small clinical studies have shown the potential of an amphotericin B-rifampin combination to improve outcomes in keratomycosis (20, 21). This combination therapy may be an option for patients with amphotericin B-nonsusceptible keratomycosis. The aim of this study was to investigate the potentiation of the antifungal activity of amphotericin B by rifampin with clinically relevant concentrations against FSSC and AFSC isolates *in vitro*.

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TABLE 1 *In vitro* susceptibilities of ocular *Fusarium solani* species complex and *Aspergillus flavus* species complex isolates to amphotericin B and rifampin alone and in combination

Organism (no. of isolates) or agent[a]	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Fusarium solani species complex (51)			
AMB	0.5–16	2	4
RIF	4,096	4,096	4,096
AMB-RIF(4)[b]	0.25–4	1	2
AMB-RIF(8)[c]	0.125–2	0.5	2
AMB-RIF(16)[d]	0.063–1	0.25	0.5
AMB-RIF(32)[e]	0.063–1	0.25	0.5
Aspergillus flavus species complex (44)			
AMB	1–32	2	4
RIF	4,096	4,096	4,096
AMB-RIF(4)[b]	0.5–4	1	2
AMB-RIF(8)[c]	0.125–2	1	2
AMB-RIF(16)[d]	0.063–1	0.25	0.5
AMB-RIF(32)[e]	0.031–1	0.25	0.5

[a]AMB, amphotericin B; RIF, rifampin.
[b]Amphotericin B at 10 concentrations in combination with 4 μg/ml rifampin.
[c]Amphotericin B at 10 concentrations in combination with 8 μg/ml rifampin.
[d]Amphotericin B at 10 concentrations in combination with 16 μg/ml rifampin.
[e]Amphotericin B at 10 concentrations in combination with 32 μg/ml rifampin.

Fifty-one FSSC and 44 AFSC strains isolated from patients with keratomycosis from the Henan Eye Institute in China were investigated. These isolates were identified based on morphology by standard methods (22). *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as quality controls for each test.

Antifungal susceptibility was assayed by the microdilution method standardized by CLSI M38-A2 (23). The isolates were tested for susceptibility to amphotericin B (Amresco E437, USA; 298 μg/mg) alone, rifampin (Alfa Aesar; 99%) alone, and amphotericin B-rifampin combinations. For antimicrobial agents alone, final concentrations ranged from 0.0313 to 16 μg/ml for amphotericin B and from 4 to 2,048 μg/ml for rifampin. Drug interactions were tested using a limited checkerboard microdilution method with combinations of rifampin at 4, 8, 16, and 32 μg/ml with 0.0313 to 16 μg/ml of amphotericin B (24, 25). The MIC was determined as the lowest concentration that prevented any discernible growth. The isolates were classified as wild type or non-wild type according to the CLSI epidemiological cutoff values (ECVs) (97.5%) proposed for amphotericin B against AFSC and FSSC, which are 4 and 8 μg/ml, respectively (26, 27). Interaction was determined by calculating the fractional inhibitory concentration index (FICI) with standard definitions: synergy FICI is $\leq$0.5, indifference FICI is $>$0.5 and $\leq$4, and antagonism FICI is $>$4 (24, 25).

The MIC_{50}, MIC_{90}, and MIC range for drugs alone and in combinations were calculated for the isolates with the SPSS statistical package (version 17.0). For calculations, any high off-scale MIC was converted to the next higher concentration.

The *in vitro* activities of amphotericin B alone, rifampin alone, and the amphotericin B-rifampin combinations against the ocular pathogens are summarized in Table 1. Ninety-eight percent of the FSSC and 97.7% of the AFSC isolates showed MICs of $\leq$8 μg/ml and MICs of $\leq$4 μg/ml, respectively, and were categorized as wild type for amphotericin B. A total of 66.7% of the FSSC and 77.3% of the AFSC isolates exhibited MICs of $\geq$2 μg/ml for amphotericin B. Upon combination with 4, 8, 16, or 32 μg/ml rifampin, the activity of amphotericin B against FSSC was 2 to 4 times, 4 to 8 times, 8 to 16 times, or 8 to 16 times greater, respectively, than that of amphotericin B alone, and the activity of amphotericin B against AFSC was 2 to 8 times, 4 to 16 times, 8 to 32 times, or 16 to 32 times greater, respectively, than that of amphotericin B alone. For a non-wild-type FSSC isolate, the MIC of amphotericin B was reduced from 16 μg/ml to 4, 1, 0.5, or 0.25 μg/ml in combination with 4, 8, 16, or 32 μg/ml rifampin, respectively. For a non-wild-type AFSC isolate, the MIC of amphotericin B was reduced from 32

TABLE 2 Interactions of amphotericin B-rifampin combinations against ocular *Fusarium solani* species complex and *Aspergillus flavus* species complex isolates

Combination	FICI and % of isolates showing interaction[a]					
	Fusarium solani species complex ($n = 51$)			*Aspergillus flavus* species complex ($n = 44$)		
	FICI (mean ± SD)	Synergy	Indifference	FICI (mean ± SD)	Synergy	Indifference
AMB-RIF(4)[b]	0.58 ± 0.24	11.8	88.2	0.61 ± 0.27	13.6	86.4
AMB-RIF(8)[c]	0.38 ± 0.19	51.0	49.0	0.42 ± 0.21	45.5	54.5
AMB-RIF(16)[d]	0.23 ± 0.19	90.2	9.8	0.19 ± 0.20	93.2	6.8
AMB-RIF(32)[e]	0.19 ± 0.11	94.1	5.9	0.17 ± 0.20	95.5	4.5

[a]Interactions: synergy, FICI of ≤0.5; indifference, FICI of >0.5 and ≤4; antagonism, FICI of >4.
[b]Amphotericin B at 10 concentrations in combination with 4 μg/ml rifampin.
[c]Amphotericin B at 10 concentrations in combination with 8 μg/ml rifampin.
[d]Amphotericin B at 10 concentrations in combination with 16 μg/ml rifampin.
[e]Amphotericin B at 10 concentrations in combination with 32 μg/ml rifampin.

μg/ml to 2, 1, 0.25, or 0.25 μg/ml in combination with 4, 8, 16, or 32 μg/ml rifampin, respectively. Table 2 summarizes the *in vitro* interactions determined by the FICI of the isolates for the amphotericin B-rifampin combinations. Antagonism was never observed for the amphotericin B-rifampin combinations.

This study has two major findings. (i) The interactions between amphotericin B and rifampin at 4, 8, 16, or 32 μg/ml were synergistic for 11.8%, 51.0%, 90.2%, or 94.1% of the FSSC isolates, respectively, and for 13.6%, 45.5%, 93.2%, or 95.5% of the AFSC isolates, respectively,. (ii) The amphotericin B-rifampin combinations may be more effective in the non-wild-type strains than in the wild-type strains against FSSC and AFSC isolates. The values of this interaction would be a potentiation of antifungal action, an effect against secondary bacterial infection, and a reduction in eye toxicity, i.e., (i) non-wild-type strains for amphotericin B alone may be susceptible to the drug combination; (ii) broad-spectrum antibacterial rifampin may be needed to combat secondary bacterial infection; and (iii) in the treatment of wild-type strains, the dosage of amphotericin B may be decreased, thereby avoiding toxicity.

In keratomycosis, Shapiro et al. demonstrated that a lower MIC is significantly associated with a good outcome (28). Lalitha et al. demonstrated that a higher MIC is associated with increased odds of perforation (9). Although the CLSI ECVs (97.5%) proposed for amphotericin B against AFSC and FSSC are 4 and 8 μg/ml, respectively, several documents indicate that isolates with MICs of ≥2 μg/ml are nonsusceptible to amphotericin B therapy and are, therefore, referred to as amphotericin B-nonsusceptible isolates (13, 15, 29). Our *in vitro* data support the clinical experience that the FSSC and AFSC are often refractory to amphotericin B. Indeed, 66.7% of FSSC isolates and 77.3% of AFSC isolates exhibited amphotericin B MICs of ≥2 μg/ml in this study. These levels are not reliably achieved in cornea in a bioactive form with the present dosage regimens of amphotericin B. The results from the pharmacokinetics of amphotericin B show that the peak concentrations of amphotericin B in debrided rabbit corneas are 6.13 μg/g (about 0.43 μg/g in a bioactive form) after one 20-μl drop of amphotericin B 0.15% is topically applied and 21.1 μg/g (about 1.48 μg/g in a bioactive form) after one 20-μl drop of amphotericin B 0.15% every 5 min for 13 applications (30). The amounts measured in the study are total drug levels. They do not indicate the quantity that is bioavailable. The studies in rabbit eyes have shown that only 7% of total amphotericin B measured in the corneas is in a bioactive form (31). For susceptible organisms, the peak concentration of amphotericin B in a bioactive form in corneas is still higher than the MIC of amphotericin B and this is still an adequate level; for nonsusceptible organisms, this level is lower than the MIC of amphotericin B and cannot exert its antifungal effect. Therefore, reducing the MIC of amphotericin B by amphotericin B-rifampin combination may be one of the keys to improving the efficacy of amphotericin B.

Rifampin is ineffective against fungi. Rifampin's mechanism of resistance in fungal cells may be related to its inability to penetrate the fungal cell membrane. Our findings suggested that the amphotericin B-rifampin combinations produced potently synergistic action against FSSC and AFSC *in vitro*. As amphotericin B acts mainly to bind to ergosterol in the fungal cell membrane and increase the membrane's permeability (32), we hypothesize for amphotericin B-susceptible strains that this increased permeability allows the intracellular substances to pass through the membrane, and this either inhibits the growth of the organism or kills it. However, for amphotericin B-nonsusceptible strains, this increased permeability of the cell membrane may not allow enough of the intracellular substances to pass through the membrane but may increase permeability enough to allow the penetration of rifampin. Once inside cells, rifampin can exert its antimicrobial effect by blocking the DNA-dependent RNA polymerase subunit B (RpoB) (33). By means of this mechanism, the activity of amphotericin B is potentiated by rifampin against fungi.

Two small clinical studies have indicated that compound amphotericin B, which mainly includes amphotericin B and rifampin, is used successfully to treat patients with keratomycosis (20, 21). Although such studies provide limited scientific evidence, they show clear clinical improvement in amphotericin B-rifampin combinations. Our findings of *in vitro* synergy between amphotericin B and rifampin support these observations. Since the concentrations of rifampin that are effective in *in vitro* combinations in this study may be achievable in cornea (34), the amphotericin B-rifampin combination may represent an attractive perspective for developing new management strategies for keratomycosis. Rifampin has been used in humans for the long-term treatment of trachoma with no serious adverse effects (35, 36). Considering that rifampin is a well-known secure drug and since no antagonism was seen between amphotericin B and rifampin in this study, we believe the amphotericin B-rifampin combination would be a well-tolerated and effective therapy form for FSSC and AFSC keratitis in humans.

In summary, the amphotericin B-rifampin combination significantly enhanced the antifungal activity of amphotericin B against FSSC and AFSC isolates. Our results suggest that an amphotericin B-rifampin combination therapy may also be beneficial for treating keratomycosis and deserves *in vivo* studies.

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REFERENCES

1. Xuguang S, Zhixin W, Zhiqun W, Shiyun L, Ran L. 2007. Ocular fungal isolates and antifungal susceptibility in northern China. Am J Ophthalmol 143:131–133. https://doi.org/10.1016/j.ajo.2006.09.042.
2. Xie L, Dong X, Shi W. 2001. Treatment of fungal keratitis by penetrating keratoplasty. Br J Ophthalmol 85:1070–1074. https://doi.org/10.1136/bjo.85.9.1070.
3. Zhong W, Sun S, Zhao J, Shi W, Xie L. 2007. Retrospective study of suppurative keratitis in 1054 patients. Zhonghua Yan Ke Za Zhi 43:245–250. (In Chinese.)
4. Xu Y, Gao C, Li X, He Y, Zhou L, Pang G, Sun S. 2013. *In vitro* antifungal activity of silver nanoparticles against ocular pathogenic filamentous fungi. J Ocul Pharmacol Ther 29:270–274. https://doi.org/10.1089/jop.2012.0155.
5. Xu Y, Pang G, Zhao D, Gao C, Zhou L, Sun S, Wang B. 2010. *In vitro* activity of thimerosal against ocular pathogenic fungi. Antimicrob Agents Chemother 54:536–539. https://doi.org/10.1128/AAC.00714-09.
6. Xie L, Zhai H, Zhao J, Sun S, Shi W, Dong X. 2008. Antifungal susceptibility for common pathogens of fungal keratitis in Shandong Province, China. Am J Ophthalmol 146:260–265. https://doi.org/10.1016/j.ajo.2008.04.019.
7. Oechsler RA, Feilmeier MR, Miller D, Shi W, Hofling-Lima AL, Alfonso EC. 2013. Fusarium keratitis: genotyping, *in vitro* susceptibility and clinical outcomes. Cornea 32:667–673. https://doi.org/10.1097/ICO.0b013e318277ac74.
8. Lalitha P, Sun CQ, Prajna NV, Karpagam R, Manoharan G, O'Brien KS, Cevallos V, McLeod SD, Acharya NR, Lietman TM. 2014. *In vitro* susceptibility of filamentous fungal isolates from a corneal ulcer clinical trial. Am J Ophthalmol 157:318–326. https://doi.org/10.1016/j.ajo.2013.10.004.
9. Lalitha P, Prajna NV, Oldenburg CE, Srinivasan M, Krishnan T, Mascarenhas J, Vaitilingam CM, McLeod SD, Zegans ME, Porco TC, Acharya NR, Lietman TM. 2012. Organism, minimum inhibitory concentration, and outcome in a fungal corneal ulcer clinical trial. Cornea 31:662–667. https://doi.org/10.1097/ICO.0b013e31823f8ae0.

10. Nayak N, Satpathy G, Prasad S, Titiyal JS, Pandey RM, Vajpayee RB. 2011. Molecular characterization of drug-resistant and drug-sensitive *Aspergillus* isolates causing infectious keratitis. Indian J Ophthalmol 59:373–377. https://doi.org/10.4103/0301-4738.83614.
11. Thomas PA. 2003. Current perspectives on ophthalmic mycoses. Clin Microbiol Rev 16:730–797. https://doi.org/10.1128/CMR.16.4.730-797.2003.
12. Loh AR, Hong K, Lee S, Mannis M, Acharya NR. 2009. Practice patterns in the management of fungal corneal ulcers. Cornea 28:856–859. https://doi.org/10.1097/ICO.0b013e318199fa77.
13. Marangon FB, Miller D, Giaconi JA, Alfonso EC. 2004. *In vitro* investigation of voriconazole susceptibility for keratitis and endophthalmitis fungal pathogens. Am J Ophthalmol 137:820–825. https://doi.org/10.1016/j.ajo.2003.11.078.
14. Tu EY, McCartney DL, Beatty RF, Springer KL, Levy J, Edward D. 2007. Successful treatment of resistant ocular fusariosis with posaconazole (SCH-56592). Am J Ophthalmol 143:222–227. https://doi.org/10.1016/j.ajo.2006.10.048.
15. Edelstein SL, Akduman L, Durham BH, Fothergill AW, Hsu HY. 2012. Resistant *Fusarium* keratitis progressing to endophthalmitis. Eye Contact Lens 38:331–335. https://doi.org/10.1097/ICL.0b013e318235c5af.
16. Gorscak JJ, Ayres BD, Bhagat N, Hammersmith KM, Rapuano CJ, Cohen EJ, Burday M, Mirani N, Jungkind D, Chu DS. 2007. An outbreak of *Fusarium* keratitis associated with contact lens use in the northeastern United States. Cornea 26:1187–1194. https://doi.org/10.1097/ICO.0b013e318142b932.
17. Thomas PA. 2003. Fungal infections of the cornea. Eye 17:852–862. https://doi.org/10.1038/sj.eye.6700557.
18. Hu S, Fan VC, Koonapareddy C, Du TT, Asbell PA. 2007. Contact lens-related *Fusarium* infection: case series experience in New York City and review of fungal keratitis. Eye Contact Lens 33:322–328. https://doi.org/10.1097/ICL.0b013e3180645d17.
19. Lalitha P, Shapiro BL, Loh AR, Fothergill AW, Prajna NV, Srinivasan M, Oldenburg CE, Quigley DA, Chidambaram JD, McLeod SD, Acharya NR, Lietman TM. 2011. Amphotericin B and natamycin are not synergistic *in vitro* against *Fusarium* and *Aspergillus* spp. isolated from keratitis. Br J Ophthalmol 95:744–745. https://doi.org/10.1136/bjo.2010.195214.
20. He M, Li Y, Lin J. 2006. Analysis of the therapeutic effect of fluconazole and compound amphotericin B on fungal corneal ulcer. Guangzhou Med J 37:56–57.
21. Fan L. 2011. Successful treatment of fungal keratitis with the compound amphotericin B. Chin Commun Doc 13:143.
22. Wang DL (ed). 2005. Medical mycology—guide to laboratory examination, p 4–37, 264–374, 421–446. People's Medical Publishing House, Beijing, China.
23. Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard—2nd ed. CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
24. Cuenca-Estrella M. 2004. Combinations of antifungal agents in therapy–what value are they? J Antimicrob Chemother 54:854–869. https://doi.org/10.1093/jac/dkh434.
25. Mukherjee PK, Sheehan DJ, Hitchcock CA, Ghannoum MA. 2005. Combination treatment of invasive fungal infections. Clin Microbiol Rev 18:163–194. https://doi.org/10.1128/CMR.18.1.163-194.2005.
26. Espinel-Ingroff A, Cuenca-Estrella M, Fothergill A, Fuller J, Ghannoum M, Johnson E, Pelaez T, Pfaller MA, Turnidge J. 2011. Wild-type MIC distributions and epidemiological cutoff values for amphotericin B and *Aspergillus* spp. for the CLSI broth microdilution method (M38-A2 document). Antimicrob Agents Chemother 55:5150–5154. https://doi.org/10.1128/AAC.00686-11.
27. Espinel-Ingroff A, Colombo AL, Cordoba S, Dufresne PJ, Fuller J, Ghannoum M, Gonzalez GM, Guarro J, Kidd SE, Meis JF, Melhem TM, Pelaez T, Pfaller MA, Szeszs MW, Takahaschi JP, Tortorano AM, Wiederhold NP, Turnidge J. 2015. International evaluation of MIC distributions and epidemiological cutoff value (ECV) definitions for *Fusarium* species identified by molecular methods for the CLSI broth microdilution method. Antimicrob Agents Chemother 60:1079–1084. https://doi.org/10.1128/AAC.02456-15.
28. Shapiro BL, Lalitha P, Loh AR, Fothergill AW, Prajna NV, Srinivasan M, Kabra A, Chidambaram J, Acharya NR, Lietman TM. 2010. Susceptibility testing and clinical outcome in fungal keratitis. Br J Ophthalmol 94:384–385. https://doi.org/10.1136/bjo.2009.158675.
29. Arendrup MC, Cuenca-Estrella M, Lass-Florl C, Hope WW. 2012. EUCAST technical note on *Aspergillus* and amphotericin B, itraconazole, and posaconazole. Clin Microbiol Infect 18:e248–e250. https://doi.org/10.1111/j.1469-0691.2012.03890.x.
30. O'Day DM, Head WS, Robinson RD, Clanton JA. 1986. Corneal penetration of topical amphotericin B and natamycin. Curr Eye Res 5:877–882. https://doi.org/10.3109/02713688609029240.
31. O'Day DM, Head WS, Robinson RD, Clanton JA. 1986. Bioavailability and penetration of topical amphotericin B in the anterior segment of the rabbit eye. J Ocul Pharmacol 2:371–378. https://doi.org/10.1089/jop.1986.2.371.
32. Groll AH, Walsh TJ. 2002. Antifungal chemotherapy: advances and perspectives. Swiss Med Wkly 132:303–311.
33. Jamieson FB, Guthrie JL, Neemuchwala A, Lastovetska O, Melano RG, Mehaffy C. 2014. Profiling of *rpoB* mutations and MICs for rifampin and rifabutin in *Mycobacterium tuberculosis*. J Clin Microbiol 52:2157–2162. https://doi.org/10.1128/JCM.00691-14.
34. Wu W, He MF, Tang XL, Wang YD. 2010. Pharmacokinetics of amphotericin B in rabbit cornea. Chin J Clin Pharmacol 26:424–426.
35. Dawson CR, Hoshiwara I, Daghfous T, Messadi M, Vastine DW, Schachter J. 1975. Topical tetracycline and rifampin therapy of endemic trachoma in Tunisia. Am J Ophthalmol 79:803–811. https://doi.org/10.1016/0002-9394(75)90740-0.
36. Darougar S, Viswalingam N, El-Sheikh H, Hunter PA, Yearsley P. 1981. A double-blind comparison of topical therapy of chlamydial ocular infection (TRIC infection) with rifampicin or chlortetracycline. Br J Ophthalmol 65:549–552. https://doi.org/10.1136/bjo.65.8.549.