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EXPERIMENTAL THERAPEUTICS

Efficacy of Voriconazole, Isavuconazole, Fluconazole, and Anidulafungin in the Treatment of Emerging *Candida auris* Using an Immunocompromised Murine Model of Disseminated Candidiasis

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ABSTRACT Antifungal activity of anidulafungin, voriconazole, isavuconazole, and fluconazole in the treatment of *Candida auris* was determined *in vitro* and *in vivo*. MICs for anidulafungin, voriconazole, isavuconazole, fluconazole, and amphotericin B were 0.5, 1, >64, 0.25, and 4 μg/ml, respectively. Significant *in vivo* efficacy was observed in the anidulafungin- and voriconazole-treated groups in survival and reduction in kidney tissue fungal burden compared to that in the untreated group (*P* values of <0.001 and 0.044, respectively). Our data showed that anidulafungin and voriconazole had comparable efficacies against *C. auris*, whereas isavuconazole did not show significant *in vivo* activity.

KEYWORDS voriconazole, isavuconazole, fluconazole, anidulafungin, *Candida auris*, disseminated candidiasis, murine model, *in vitro*, *in vivo*, multidrug resistance

Candida auris is an emerging infection that was first described in 2009, followed by multiple reports from countries around the world, including the United States (1–3). *C. auris* poses a challenge in both identification and effective treatment caused by its unique characteristics and the rather complex identification tools necessary, making it a global concern (4–8). *C. auris* is a multidrug-resistant (MDR) organism, with emergence of pan-resistant strains, characterized by reduced susceptibility to the three main antifungal groups (azoles, polyenes, and echinocandins), thereby making *C. auris* one of the most difficult pathogens to treat of all clinically relevant *Candida* species (9–11). This increases the need to identify drugs that are efficacious against this pathogen. In the current study, we evaluated the *in vitro* activity and efficacy of a number of antifungal agents, including anidulafungin (ANID), voriconazole (VOR), isavuconazole (ISA), and fluconazole (FLU), in the treatment of *C. auris* using an immunocompromised murine model of disseminated candidiasis.

A clinical isolate of *C. auris* (MRL 35368) known to be infective (12–14) was used in this study. Susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) document M27 (15). After 24 h of incubation, the MICs for ANID, FLU, ISA, amphotericin B (AMB), and VOR were 0.5, >64, 0.25, 4, and 1 μg/ml, respectively. Using tentative *C. auris* breakpoints suggested by the CDC (16), this clinical isolate is resistant to AMB and FLU.

In vivo testing was performed using a previously described disseminated *C. auris* infection model (12–14). All procedures were performed in compliance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* (17) and with approval of the Case Western Reserve University Institutional Animal Care and Use Committee (IACUC).

Citation Gamal A, Long L, Herrada J, Aram J, McCormick TS, Ghannoum MA. 2021. Efficacy of voriconazole, isavuconazole, fluconazole, and anidulafungin in the treatment of emerging *Candida auris* using an immunocompromised murine model of disseminated candidiasis. Antimicrob Agents Chemother 65:e00549-21. https://doi.org/10.1128/AAC.00549-21.

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Received 19 March 2021
Returned for modification 31 May 2021
Accepted 28 June 2021
Accepted manuscript posted online 6 July 2021
Published 17 August 2021

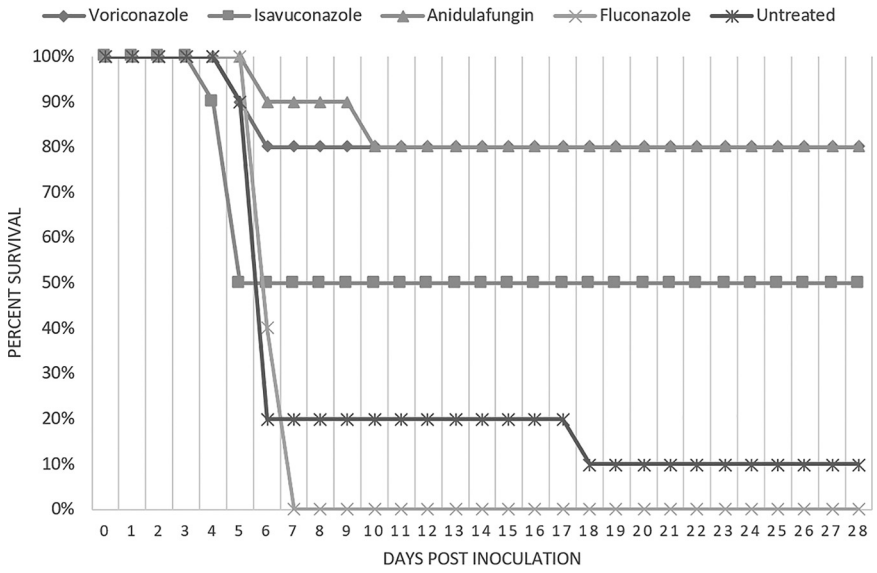

FIG 1 Effects of various antifungals compared to untreated controls on the survival of mice infected with *C. auris*.

Female BALB/c mice ($n = 15$) (weighing ~20 g; Charles River Laboratories, Wilmington, MA) were used in the study. Treatment was initiated 2 h postchallenge. Treatment groups consisted of twice a day dosing of (i) ANID at 12 mg/kg of body weight intraperitoneally (i.p.), (ii) VOR at 12 mg/kg i.p., (iii) ISA at 20 mg/kg per os (p.o.), or (iv) FLU at 20 mg/kg p.o. Untreated control animals were also included; 10 animals were used to assess survival, while 5 animals were used to assess the effect on tissue fungal burden per group. Efficacy endpoints used were animal survival and kidney and brain fungal load.

Survival was monitored for 28 days postinoculation (Fig. 1). The animals treated with VOR and ANID at 12 mg/kg showed the highest survival rates (80% and 90%, respectively) at day 7 postinoculation and showed a survival rate of 80% for both drugs at day 28 postinoculation, which is significantly prolonged compared to that of the untreated control group, for which only 10% survival was observed at day 28 (P values of 0.009 and 0.005, respectively). Additionally, the VOR- and ANID-treated groups demonstrated significantly better survival rates than the FLU-treated group (P value of <0.001). The group treated with ISA at 20 mg/kg showed an average survival rate of 50%, while the group treated with FLU at 20 mg/kg showed the lowest percent survival (0% by day 7 postinoculation).

A subgroup of animals ($n = 5$) were euthanized on day 8 postinfection. Kidneys were removed aseptically, weighed, homogenized in 1 ml of phosphate-buffered saline (PBS), serially diluted, and then plated onto potato dextrose agar (Becton, Dickinson and Company, Sparks, MD) and cultured at 37°C for 48 h to determine the fungal tissue burden, expressed as CFU per gram of tissue. Efficacy of antifungal agents was evaluated as a reduction in $\log_{10}$ CFU compared with those of control and other tested groups.

Table 1 shows the tissue fungal burden in the kidneys and the brains of mice challenged with *C. auris* (expressed as average log CFU per gram $\pm$ standard deviation [SD]). As expected, the mice in the untreated control group showed the highest tissue fungal burden (8.75 ± 0.6 and 5.92 ± 0.5 average log CFU/g for kidneys and brain, respectively). The ANID- and VOR-treated groups showed significant reductions in the kidney tissue fungal burden (2.90 ± 0.5 and 5.36 ± 3.0 average log CFU/g, respectively) compared to that of the untreated group (P value < 0.001 and P value $= 0.044$, respectively). Moreover, there was no significant difference between ANID and VOR (P value $= 0.296$). Additionally, the ANID-treated group demonstrated a significant reduction in kidney fungal burden

TABLE 1 Effects of antifungals on kidney and brain tissue fungal burdens compared to those in the untreated control

Group	Kidney		Brain	
	Avg log CFU ± SD	*P* value vs untreated	Avg log CFU ± SD	*P* value vs untreated
Untreated control	8.75 ± 0.6		5.92 ± 0.5	
Anidulafungin	2.90 ± 0.5	<0.001	4.62 ± 0.3	0.421
Voriconazole	5.36 ± 3.0	0.044	4.26 ± 1.3	0.118
Fluconazole	6.76 ± 1.8	0.73	5.14 ± 1.5	1.000
Isavuconazole	7.25 ± 0.3	1.000	5.36 ± 0.2	1.000

compared to those of the FLU-treated group and ISA-treated group (6.76 ± 1.8 and 7.25 ± 0.3 average log CFU/g ± SD, respectively; *P* values = 0.009 and 0.016, respectively). On the other hand, FLU and ISA did not exhibit a significant reduction in tissue fungal burden in the kidneys compared to that in the untreated group (*P* values of 0.73 and 1.00, respectively)

Finally, none of the of the tested compounds demonstrated significant reduction in brain tissue fungal burden compared to that in untreated controls (*P* values = 0.421, 0.118, 1.00, and 1.00 for ANID, VOR, FLU, and ISA, respectively). This suggests poor central nervous system (CNS) penetration of the tested agents.

Evaluation of the *in vitro* activity of echinocandins against the MDR *C. auris* in different studies, which included larger numbers of strains, showed that these agents are active against most *C. auris* strains (18, 19). However, some strains were found to show resistance even to this class of antifungals, especially those with an S639F mutation in *FKS1* hot spot region 1 (18, 19). In our study, we confirmed that ANID showed the greatest effect in reduction of tissue fungal burden as well as potent *in vitro* activity against a highly infective clinical isolate of *C. auris*. In the present study, ANID showed a significant effect through reduction of kidney fungal tissue burden. However, unlike APX001A/APX001 (fosmanogepix), which significantly reduced brain tissue fungal burden (13), ANID failed to do so, suggesting that it may have poor CNS penetration compared to that of fosmanogepix. However, since different *C. auris* strains were used in these studies, evaluating the efficacies of different agents in a head-to-head comparison is not possible. Recently, echinocandin-resistant *C. auris* has been reported in a number of cases (20, 21); however, some promising newly developed antifungals have shown activity against these strains (19, 22–24).

Interestingly, VOR showed significant reduction of tissue fungal burden in kidneys, which was not the case for ISA, as ISA demonstrated the highest *in vitro* activity of the three azoles tested but failed to exhibit significant reduction of tissue fungal burden *in vivo* compared to that in the untreated group. This may be explained by the fact that ISA is highly bound to plasma proteins and achieves a lower maximum plasma concentration and therefore less tissue penetration in animal models than VOR (25, 26). However, the relationship between tissue concentration and efficacy of VOR was reported as variable in various studies (27, 28), which may be caused by distribution of the drug to the wrong subcompartment, lack of bioavailability, or accumulation (in tissue) at a concentration below the threshold required for activity (29). Additionally, ISA failed to demonstrate noninferiority relative to caspofungin (CAS) in the treatment of candidemia and invasive *Candida* infections (30).

In conclusion, ANID showed potent *in vitro* and *in vivo* activities against a clinical isolate of *C. auris* (MRL 35368) which demonstrate resistance to FLU, as well as AMB. VOR has activity against this isolate at levels comparable to that of ANID. Although ANID and VOR were effective in reducing tissue fungal burden *in vivo* in kidneys, they were less active in clearing brain infection. Further work is needed to determine factors that play a role in the ability of an antifungal to penetrate various tissues, including the CNS, and

effectively eliminate *C. auris*. These studies may lead to the development of drugs that can act effectively in various tissues. Additionally, further work is necessary to evaluate the variation observed among different azoles used against *C. auris*. Finally, confirmation of these studies using additional clinical isolates of *C. auris* is recommended.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institutes of Health (grant number R21 AI143305-01).

In compliance with the ICMJE uniform disclosure form, Mahmoud Ghannoum declares that he received funding from Pfizer, Scynexis, Inc., Cidara Therapeutics, and Amplyx Pharmaceuticals. All other authors have declared that there are no other relationships or activities that could appear to have influenced this work.

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