P060
A preliminary in vitro and in vivo evaluation of the effect and action mechanism of 17-AAG combined with azoles against azole-resistant Candida spp.

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Intensive candidiasis is the primary reason for the increasing cases of mortality in a medical environment. The resistance spectra of Candida species to antifungal drugs, among which Candida auris is the most prominent, have gradually expanded.

Objective: Hsp90 plays a protective role in the stress response of fungi and facilitates their virulence. In contrast, Hsp90 inhibitors can improve the resistance of fungi to antifungal drugs by regulating the heat resistance of Hsp90 and thereby destroying the integrity of the fungal cell wall. Therefore, we used Hsp90 inhibitor in combination with different antifungal drugs to explore its antifungal effect and mechanism.

Methods: The drugs tested for the resistance included itraconazole, voriconazole, posaconazole, fluconazole, and 17-AAG. A total of 20 clinical strains of Candida were investigated. The broth microdilution checkerboard technique, as adapted from the CLSI M27-A4 method, was applied in this study. At the same time, the effect of 17-AAG combined with antifungal drugs on the formation of Candida biofilm was observed, and the animal experiment of C. albicans was carried out in vivo. Moreover, we determined that with the use of rhodamine 6 G to detect drug efficacy and that of dihydrorhodamine-123 to detect intracellular reactive oxygen species (ROS).

Results: We found that 17-AAG alone exerted limited antifungal activity against all tested strains. The MIC range of 17-AAG was 8 to >32 μg/ml. The synergy among 17-AAG and voriconazole, voriconazole, and posaconazole was observed against 10 (50%), 7 (35%), and 1 (4%) of all isolates, respectively. Moreover, the synergy between 17-AAG and Voriconazole was observed against 1 (50%) strains of azole-resistant Candida. However, no antagonism was recorded. In in vitro, the combination group also significantly prolonged the infection event and improved the survival of larvae. Treatment with 17-AAG combined with azole drugs inhibited the efflux pump of fungi and promoted the accumulation of ROS in the fungal cells.

Conclusion: Our result adequately verifies the influence of 17-AAG on the formation of Candida spp. biofilms. The mechanism of 17-AAG combined with azole could kill fungi by inhibiting drug efflux and increasing intracellular reactive oxygen species. These results thereby provide a new idea to further explore drugs against drug-resistant Candida spp.

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Identification of Octenidine (dihydrochloride) inhibiting fungal filamentation by the repressing approach

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Objective: Hyphal formation is an important virulence factor of opportunistic pathogenic fungi and plays a vital role in invasive fungal infections. Therefore, hyphas can act as a specific target against invasive fungal infections and it is a new attempt to focus on identification of compounds inhibiting hyphal growth. Amphotericin B has potent inhibitory effect on the growth of hyphae, but its high toxicity limits its clinical use. To address this question, we performed a high-throughput screen of an FDA-approved compound library (HTS-L022, MCE®) to identify compounds with mycelial inhibitory function.

Methods: We performed a high-throughput screen of an FDA-approved compounds library to identify potentially novel compounds for inhibiting hyphal growth and used amphotericin B (1.5 μM) as a positive control drug. The screening schematic is shown in Figure 1a. Firstly, we investigated the mycelial inhibitory activity of each compound at 100 μM in RPMI 1640 medium with Candida albicans SN152 strain. Secondly, micro-chip-based dilution method was applied to determine the minimum hyphas-inhibiting concentration of compounds. The compound being included in the next round could inhibit the hyphal formation when its concentration was ≤12.5 μM. Lastly, we expanded mimdans to YPD containing 10% FBS, YPD containing 5 μg/mL nystatin (GlcNAc), and Spender medium. The final candidate compound was determined due to its minimum hyphas-inhibiting concentration was ≤3.125 μM in four mediums.

Results: We screened a library of FDA-approved compound and identified 117 Candida compounds that inhibit hyphal growth (<100 μM). We excluded 14 compounds with known antifungal activity, and finally 103 compounds were included in the next step of mycelial inhibitory activity screening (Fig. 1a). We further identified that 14 of 103 Candida compounds (red square) could significantly inhibit the growth of mycelia at a concentration not higher than 3.125 μM in RPMI 1640 medium (Fig. 1b). We then expanded the types of media that induce mycelial growth (such as YPD medium with 10% FBS, YPD medium with 5 μg/mL GlcNAc, and Spender medium) and used amphotericin B (1.5 μM) as a positive control drug and found that Octenidine (dihydrochloride) still has a significant inhibitory effect on mycelial growth in various mycelial inhibition media when it is as low as 3.125 μM (Fig. 2a and b).

Conclusion: Our study demonstrates that Octenidine (dihydrochloride) has a potent hyphal inhibitory activity and is helpful to open the way for the development of new antifungal therapeutics targeting filamentous formation.