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Evaluation of the Antifungal Activity of TDT 067 (Terbinafine in Transfersome®) in Combination with Antifungals Used in the Treatment of Systemic Fungal Infections

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INTRODUCTION

- TDT 067 is a novel carrier-based dosage form of terbinafine in Transfersome® (1.5% spray). Transfersomes are ultradeformable lipid-based vesicles which can cross the skin permeability barrier driven by a transcutaneous water gradient.
- TDT 067 has been formulated for topical delivery of terbinafine to the nail, nail bed, and surrounding tissue and is currently being investigated in a Phase III study for the treatment of onychomycosis.
- Recent approaches being investigated for the management of invasive fungal infections, such as candidiasis and aspergillosis, include combination antifungal therapy which has the potential for additive activity compared with either agent alone.[1]

OBJECTIVES

- The effect of combining TDT 067 with caspofungin and voriconazole, used in the treatment of systemic mycoses, was evaluated against relevant fungal species to determine if addition of TDT 067 increased the antifungal activity of these agents.

METHODS

- Combination minimum inhibitory concentration (MIC) testing was performed by a checkerboard microdilution assay with two antifungal agents combined in varying *concentrations* to determine whether they had synergistic (combination has more potent antifungal activity than either drug alone), indifferent, or antagonistic (addition of a second drug raises the MIC of the first drug) interactions on the respective MICs.
- Interaction was defined by the Fractional Inhibitory Concentration Index (FICI), defined as MIC drug A in combination/MIC drug A alone + MIC drug B in combination/MIC drug B alone.
- Visually clear wells from the MIC combination assay were subcultured for colony *forming* unit (CFU) counts to determine cidality.

RESULTS

Combination of TDT 067 and caspofungin against Candida *strains*

- TDT 067 combined with caspofungin demonstrated synergy against two strains of *Candida albicans* (MICs of TDT 067 and caspofungin up to 16-fold and 62-fold lower, respectively, in combination).
- No antagonism was observed.

Table 1. Minimum inhibitory concentrations (MICs; µg/mL) and interaction of TDT 067 in combination with caspofungin against Candida *strains*

Organism	Caspofungin individual MIC	TDT067 individual MIC	Caspofungin MIC in combination	TDT067 MIC in combination	Interaction (FICI)
Candida albicans					
Strain 1	0.5	8	0.008	2	0.266
Strain 2	0.25	8	0.06	0.5	0.3025
Strain 3	0.25	4	0.06	4	1.24
Strain 4	0.25	2	0.06	1	0.74
Strain 5	0.25	4	0.12	2	0.98
Candida glabrata					
Strain 1	0.5	8	0.12	8	1.24
Strain 2	0.25	2	0.12	1	0.98
Strain 3	0.5	2	0.25	0.25	0.625
Strain 4	0.5	2	0.25	0.25	0.625
Strain 5	0.5	4	0.25	0.25	0.5625
Candida krusei					
Strain 1	0.5	0.5	0.008	0.5	1.016
Strain 2	0.5	0.5	0.008	0.5	1.016
Strain 3	0.5	0.25	0.5	0.004	1.016
Strain 4	0.5	0.5	0.125	0.5	1.25
Strain 5	0.5	1	0.25	0.125	0.625
Candida parapsilosis					
Strain 1	0.5	0.5	0.25	0.25	1
Strain 2	0.5	1	0.008	1	1.016
Strain 3	0.5	1	0.008	1	1.016
Strain 4	0.5	1	0.008	1	1.016
Strain 5	0.5	1	0.008	1	1.016

FICI, Fractional Inhibitory Concentration Index (values ≤0.5 indicate a synergistic interaction; values >0.5–≤4.0 indicate no interaction; values >4.0 indicate antagonistic interaction).

- TDT 067 combined with caspofungin resulted in ≥99.9% reduction in CFUs against all *Candida krusei* and *Candida glabrata* strains tested, and one *Candida albicans* strain (Table 2).

Table 2. Minimum fungicidal concentrations of TDT 067 in combination with caspofungin against Candida *strains: lowest concentration to exhibit ≥99.9% reduction in colony counts (MFCs; µg/mL)*

Organism	Caspofungin MFC in combination	TDT 067 MFC in combination
Candida albicans		
Strain 1	>8	>8
Strain 2	>8	>8
Strain 3	>8	>8
Strain 4	>8	>8
Strain 5	2	0.015
Candida glabrata		
Strain 1	4	4
Strain 2	0.5	0.5
Strain 3	0.5	0.016
Strain 4	0.5	0.016
Strain 5	0.5	4
Candida krusei		
Strain 1	0.5	0.066
Strain 2	0.5	0.008
Strain 3	1	0.008
Strain 4	1	0.004
Strain 5	0.5	0.008
Candida parapsilosis		
Strain 1	>8	>16
Strain 2	>8	>16
Strain 3	>8	>16
Strain 4	>8	>16
Strain 5	>8	>16

Combination of TDT 067 and voriconazole against filamentous fungi

- TDT 067 combined with voriconazole demonstrated synergistic interaction against 7/20 strains of the filamentous fungi tested, including three *Aspergillus fumigatus* strains (MICs of TDT 067 and voriconazole up to 4-fold and 16-fold lower, respectively, in combination), two *Aspergillus flavus* strains (MICs of TDT 067 and voriconazole up to 1000-fold and 4-fold lower, respectively), and two *Rhizopus* strains (MICs of TDT 067 and voriconazole up to 16-fold and 128-fold lower, respectively, in combination).
- No antagonism was observed.

Table 3. Minimum inhibitory concentrations (MICs; µg/mL) and interaction of TDT 067 in combination with voriconazole against filamentous fungi

Organism	Voriconazole individual MIC	TDT067 individual MIC	Voriconazole MIC in combination	TDT067 MIC in combination	Interaction (FICI)
Aspergillus fumigatus					
Strain 1	0.12	0.5	0.06	0.12	0.74
Strain 2	0.25	0.5	0.06	0.12	0.48
Strain 3	0.25	0.5	0.06	0.12	0.48
Strain 4	0.5	16	0.03	4	0.31
Strain 5	0.12	1	0.03	0.5	0.75
Aspergillus flavus					
Strain 1	0.5	>0.004	0.25	0.000004	0.5
Strain 2	0.25	0.016	0.12	0.001	0.54
Strain 3	0.5	0.016	0.12	0.004	0.49
Strain 4	0.5	0.03	0.12	0.016	0.77
Strain 5	0.5	0.03	0.03	0.016	0.59
Rhizopus					
Strain 1	16	15	2	4	0.39
Strain 2	16	4	2	4	1.13
Strain 3	128	4	64	2	1.00
Strain 4	64	15	8	15	1.13
Strain 5	16	8	0.125	0.5	0.07
Fusarium solani					
Strain 1	16	16	16	0.25	1.02
Strain 2	4	469	2	117	0.75
Strain 3	32	30	32	8	1.27
Strain 4	8	30	8	0.06	1.00
Strain 5	32	60	8	30	0.75

FICI, Fractional Inhibitory Concentration Index (values ≤0.5 indicate a synergistic interaction; values >0.5–≤4.0 indicate no interaction; values >4.0 indicate antagonistic interaction).

- TDT 067 combined with voriconazole resulted in ≥99.9% reduction in CFUs against all the *Aspergillus* strains tested (Table 4).

Table 4. Minimum fungicidal concentrations of TDT 067 in combination with voriconazole against filamentous fungi: lowest concentration to exhibit ≥99.9% reduction in colony counts (MFCs; µg/mL)

Organism	Voriconazole MFC in combination	TDT 067 MFC in combination
Aspergillus fumigatus		
Strain 1	0.06	0.12
Strain 2	0.06	0.12
Strain 3	0.12	0.03
Strain 4	0.12	2
Strain 5	0.03	0.5
Aspergillus flavus		
Strain 1	0.25	0.00006
Strain 2	0.12	0.001
Strain 3	0.12	0.004
Strain 4	0.12	0.016
Strain 5	0.03	0.016
Rhizopus		
Strain 1	64	8
Strain 2	>128	>30
Strain 3	>128	>30
Strain 4	>128	>30
Strain 5	>128	>15
Fusarium solani		
Strain 1	128	16
Strain 2	0.25	1875
Strain 3	>128	>60
Strain 4	>128	>60
Strain 5	64	117

CONCLUSIONS

- TDT 067 showed synergistic interactions in combination with caspofungin and voriconazole, with the greatest effect shown by the combination of TDT 067 and voriconazole against *Aspergillus* strains, resulting in fungicidal activity.
- Importantly, there was no antagonism with the combination of TDT 067 with either antifungal against any of the strains tested.
- Other preclinical studies have shown that the antimicrobial activity of TDT 067 extends to dermatophytes, yeasts, filamentous fungi, and filamentous bacteria, suggesting that TDT 067 may have broader clinical utility than onychomycosis.[2,3]
- TDT 067 may also have clinical utility in combination with antifungals used for treatment of invasive fungal infections.

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