Original Article

Effects of caspofungin sequentially combined with fluconazole on the biomass of mixed *Candida albicans* and *Candida glabrata* biofilm *in vitro*

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Abstract

Introduction: Previous study revealed that pre-exposure of *C. albicans* biofilm with fluconazole (FLU) lead to a significant decrease of the efficacy of caspofungin (CAS). This study aimed to investigate the effect of CAS sequentially combined with FLU on the reduction of mixed *Candida albicans* and *Candida glabrata* biofilm

Method: Mixed *C. albicans* and *C. glabrata* biofilms were pretreated either with CAS or FLU at 1 MIC concentration for 1 to 6 hours, then FLU or CAS at 1 MIC concentration were added to selected biofilms, respectively. Biofilm quantitation was evaluated using crystal violet (CV) assay. Additionally, antifungal activity of sequential combination drugs at subinhibitory concentrations were analyzed at 3 and 4 hours pre-exposure time.

Result: The antifungal effects of CAS/FLU combination at 1 MIC concentration against CAS-pretreated mixed biofilms at every pre-exposure time were not different, however, the inhibitory effects of combinations were significantly declined when mixed biofilms were pretreated with FLU for a longer period of time *(P<0.001)*. At 3 hours FLU-pretreatment, the percentage of biofilm reduction in response to CAS/FLU combinations was significantly increased when CAS at 0.5 MIC and FLU at 0.5 MIC were combined (39.17%), compared to that of CAS at 1 MIC combined with FLU at 1 MIC (12.52%) *(P<0.05)*.

Conclusion: The efficacy of CAS/FLU combinations treatment on the biomass of mixed *C. albicans* and *C. glabrata* biofilm was dependent on the pre-exposure time and sequence of combination drugs. Cell viability and microstructure of mixed biofilm in response to CAS/FLU sequential combination should be further evaluated.

Key word: Biofilm, *Candida albicans*, *Candida glabrata*, Caspofungin, Fluconazole

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Introduction

Candida infection is one of the most common infections in immunocompromised patients especially HIV-infected individuals and AIDS patients[1] and it has become a major problem in elderly patients.[2] Candidiasis has been attributed to *Candida albicans*; however, infections caused by non-*Candida albicans Candida* (NCAC) species, such as *Candida glabrata, Candida* tropicalis and *Candida parapsilosis* are increasingly being recognized and continuously reported.[3, 4]

Triazoles and echinocandins, two classes of antifungal agents with distinct mechanisms of action, are used as standard therapy for Candida infections, according to the guidelines recently published by Infectious Disease Society of America (IDSA).[5] Fluconazole (FLU), the main drug of choice of triazoles, disturbs the synthesis of ergosterol in cell membrane resulting in growth arrest.[6] FLU is generally effective against candidiasis but its use may be limited by the increasing prevalence of *Candida* species with acquired or intrinsic resistance.[7] Caspofungin (CAS), the first approved echinocandin, is semisynthetic amphiphilic lipopeptides that inhibits the synthesis of β-1,3-glucan, the major structural component of Candida cell walls, resulting in osmotic instability and fungal cell lysis. CAS is recommended as first-line treatment for candidemia/invasive candidiasis in all patient.[7]

Biofilms are defined as highly organized communities of microorganisms that are attached to an abiotic or biotic surface and are enclosed within a self-produced extracellular polymeric matrix.The advantages of biofilm formation include protection from the environment, resistance of physical and chemical stress, metabolic cooperation, and a community-based regulation of gene expression. *Candida* species are able to attach to polymeric surfaces and generate a biofilm structure, protecting the organisms from the host defenses and antifungal drugs.[8, 9] *Candida* biofilms play a growing role in human medicine. Indeed, *Candida* biofilms are more resistant than their planktonic counterparts to various antifungal agents including amphotericin, fluconazole, itraconazole and ketoconazole.[10 – 12]

To improve the activity against Candida biofilms and Candida biofilm-associated infections, the use of new combined antifungal agents might be more successful. In refractory cases of invasive fungal infections, combinations of triazoles and echinocandins have been studied as promising therapies to reduce high attributable mortality rates.[13] Our previous *in vitro* study showed that the biomass of mixed *C. albicans* and *C. glabrata* biofilm were significantly decreased when CAS at 0.25 MIC and FLU (0.5 or 0.25 MIC) were simultaneously combined, but those were not different when CAS at 0.5 MIC simultaneously combined with FLU (0.25 or 0.5 MIC) compared to CAS treatment alone.[11] However, *in vitro* study of sequential therapy demonstrated a higher resistance to CAS of FLU pretreated-*C. albican* biofilm at the high concentration of FLU (more than 16 μg/ml).[14] Therefore, the inhibitory effects of sequential therapy with CAS and FLU on the reduction of mixed *C. albican* and *C. glabrata* biofilm were further investigated here.

Methods

1) Organisms

C. glabrata DMST46683 and *C. albicans* ATCC10231 were kindly supported by Department of Medical Sciences, Ministry of Public Health, Thailand. Stock cultures were divided into small portions and stored at -80°C in 20% glycerol tryptone soil broth.

2) Antifungal agents

CAS was provided by Merck Sharp & Dohme Limited, and FLU was supported by Hetero Thailand Limited. Both were obtained in powder form and reconstituted in sterile distilled water to make stock solutions of 1,024 μg/ ml for FLU and 16 μg/ ml for CAS.

3) Culture condition

Candida spp. were grown in Sabouraud dextrose broth (SDB) (Difco Laboratories, Detroit, MI). Fifty milliliters of SDB medium was inoculated with a loopful of *Candida* from thawed stock cultured and incubated on the orbital shaker (Stuart, SI500, UK) at 37°C for 24 hours. Cells were harvested and counted using hematocytometer. Cells were resuspended in RPMI 1640 without sodium bicarbonate supplemented with L-glutamine and buffered with morpholinepropanesulfonic acid (MOPS) (Gibco, USA), and the suspension was adjusted to 10^6 cells/ml with RPMI 1640

4) Biofilm formation

Biofilms were performed on polystyrene, flat bottom, 96-well microtiter plates (Corning Incorporated, Corning, N.Y.) as previously described.[11] Briefly, biofilms were formed by pipetting 50 µl of each standardized cell suspensions into selected wells of the microtiter plate and incubating the plate for 24 hours at 37°C. After biofilm formation, the medium was discarded and non-adherent cells were removed by thoroughly washing the biofilms three times in sterile PBS.

5) Effect of pre-exposure time on the antifungal activity of sequential CAS/FLU combination treatment at the concentration of 1 MIC

Mature biofilms were treated with either CAS (= CAS-pretreated) or FLU (= FLU-pretreated) at 1 MIC. The concentration at 1MIC of CAS against mixed *C. albicans* and *C. glabrata* biofilm is 0.56 µg/ml, and 1 MIC of FLU is 309 µg/ml.[11] After mixed biofilms were incubated with the first drug for 1 - 6 hours, the second drug were added to the pretreated biofilm to make a CAS/FLU combination. Biofilm wells without drug were prepared for controls. The total incubation time for all was 24 hours at 37°C. Triplicated wells were included in each group for all experiment, and triplicated experiments were performed.

6) Effect of subinhibitory concentration of combinations on the reduction of pretreated mixed biofilm

After mixed biofilms were incubated with either CAS or FLU at the subinhibitory concentration for 3 hours, the second drug were added to selected biofilms to make a concentration ratio as 1 MIC_{cas} + 1 MIC_{flu}, 0.5 MIC_{cas} + 0.5 MIC_{flu}, 0.5 MIC_{cas} + 0.25 MIC_{flu}, 0.25 MIC_{cas} + 0.5 MIC_{flu}, 0.25 MIC_{cas} + 0.25 MIC_{flu}. In addition, the 4 hours-pretreated mixed biofilms were tested under the same conditions as that of the 3 hours-pretreated biofilm. Mixed biofilms treated with single drug (CAS or FLU) at the concentration of 1 MIC were also performed. All experimental and control groups were incubated for 24 hours at 37°C. Triplicated wells were included in each group for all experiment, and triplicated experiments were performed.

7) Biofilm quantitation

The quantitation of biofilm biomass was determined after 24 hours of incubation at 37°C with antifungal agents using crystal violet (CV) assay as described previously.[11] Briefly, the medium was aspirated from each well and the adherent cells were washed twice with phosphate-buffered saline (PBS). One hundred microliters of 99% methanol were added to each well and fixed for 15 minutes. Wells were air dried after methanol discarded, and 100 µl of 0.5% (w/v) CV solution was added. The excess CV was then removed after 20 minutes incubation. Finally, bound CV was released by adding 150 µl of 33% acetic acid. The acetic acid was gently pipetted to completely solubilize the CV for 1 minute, and plate was read using a microtiter plate reader (Opsys MR, Dynex, USA) at 590 nm. The antifungal effect on biofilm was measured by comparing the reduction in the mean absorbance of the antifungal-challenged biofilm to that of the unchallenged biofilm as control and expressed as the percentage of biofilm reduction following formula

$$\text{Percentage of biofilm reduction} = \{(OD_{control} - OD_{drug})/OD_{control}\} \times 100$$

8) Statistical analysis

The average values of the triplicated wells were used in the data analysis to calculate the mean ± standard deviation (SD) of all experiments performed under the same conditions. The analyses were performed by using GraphPad Prism version 5 (GraphPad Software, Inc.). Differences between mean values were assessed by Two-way analysis of variance (ANOVA) with Bonferroni post-test. A value of P <0.05 were considered statistically significant.

Results

1) Effect of sequence and varying pre-exposure time of CAS/FLU combination against mixed biofilm

Present studies demonstrated that the efficacy of CAS/FLU combination against mixed *C. albicans* and *C. glabrata* biofilms was affected by both the order of combination drug and the duration of pre-exposure time (Figure 1). Preincubation of mixed biofilm in CAS at the concentration of 1 MIC for 1 to 6 hours before FLU exposure did not alter the efficacy of CAS/FLU on biofilm reduction. However, the inhibition activity of CAS/FLU combination against mixed biofilm were continuously decreased when biofilm were pretreated with FLU for a longer period of time (1 to 4 hours) (Figure 1). The percentages of biofilm reduction in response to combinations when mixed biofilms were preincubated with FLU for 1 hour was 64.66 ± 8.07 (means ± SD), while those were significantly declined when biofilms were preincubated with FLU for 3 hours (16.07 ± 32.00), and 4 hours (-16.89 ± 50.85) (*P<0.001*).

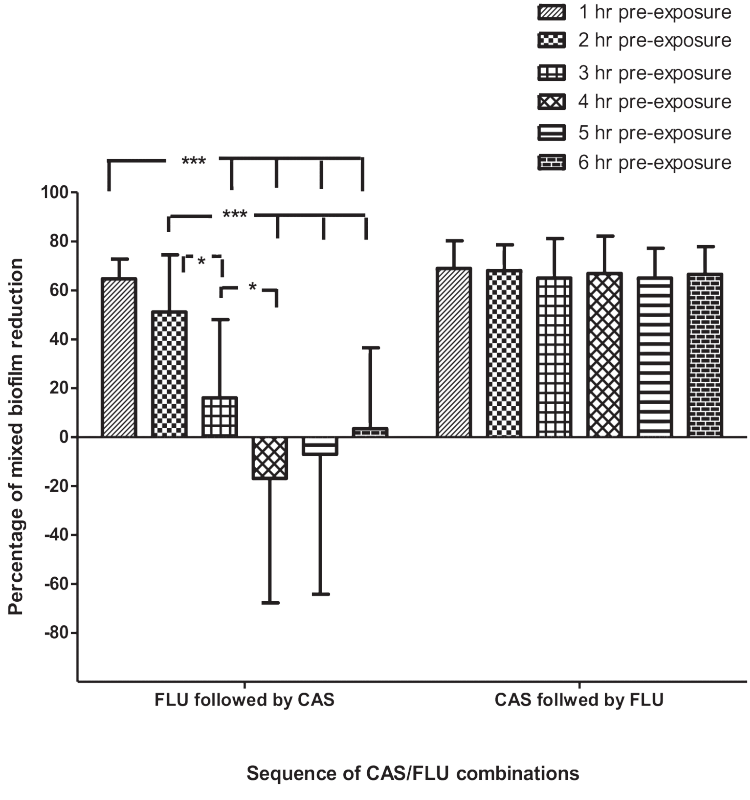

Figure 1 Assessment of biomass of mixed *Candida albicans* and *Candida glabrata* biofilms following sequential treatment with caspofungin (CAS) and fluconazole (FLU) at the concentration of 1 MIC. Each bar represents means ± SD from three experiments. * shows statistically significant difference at $P < 0.05$, *** shows statistically significant difference at $P < 0.001$ among experiment groups using two-way ANOVA with Bonferroni post-test.

2) The inhibitory effect of the pre-exposure time and subinhibitory concentration ratio of combinations against mixed biofilm

At 3 hours pre-exposure time, the percentage of biofilm reduction following treatment the FLU-pretreated biofilm with CAS at the concentration of 1 MIC (12.52 ± 17.38) was significantly reduced compared to those of CAS only (31.07 ± 11.19) (*P< 0.05*). However, this inhibitory effect was significantly increased when FLU-pretreated biofilm was combined with CAS at the concentration of 0.5 MIC (39.17 ± 7.27) compared to those at the concentration of 1 MIC (*P< 0.01*) (Figure 2). At 4 hours pre-exposure time, the percentage of biofilm reduction after treatment the FLU-pretreated biofilm with CAS either at the concentration of 1 MIC (*P<0.05*), 0.5 MIC (*P<0.001*) or 0.25MIC (*P<0.05*) were significantly decreased compared to those of single drug therapy (FLU only and CAS only) (Figure 2).

Again, preincubation of mixed biofilm with CAS, either for 3 or 4 hours, prior to FLU exposure at subinhibitory concentration (0.5 MIC, 0.25 MIC) did not change the inhibitory effects of combinations compared to those of CAS or FLU monotherapy (Figure 3).

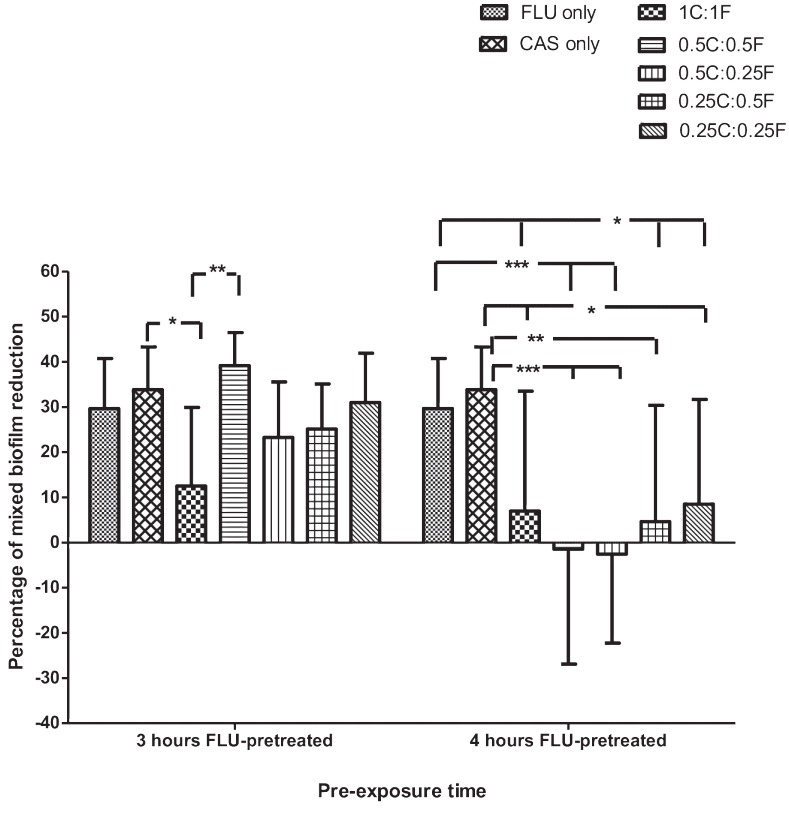

Figure 2 Assessment of biomass of fluconazole-pretreated mixed *Candida albicans* and *Candida glabrata* biofilms followed by caspofungin at the subinhibitory concentration (times of MIC). Each bar represents means ± SD from three experiments. * shows statistically significant difference at *P < 0.05*, ** shows statistically significant difference at *P < 0.01*, *** shows statistically significant difference at *P < 0.001* among experiment groups using two-way ANOVA with Bonferroni post-test. (C is abbreviated from caspofungin, F is abbreviated from fluconazole)

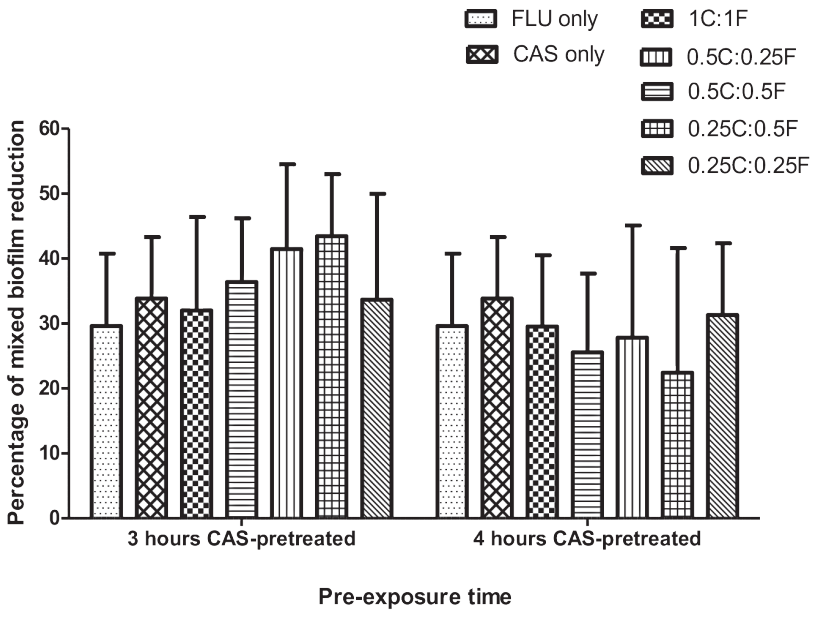

Figure 3 Assessment of biomass of caspofungin-pretreated mixed *Candida albicans* and *Candida glabrata* biofilms followed by fluconazole at the subinhibitory concentration (times of MIC). Each bar represents means ± SD from three experiments. No statistically significant difference among experiment groups within the same pre-exposure time using two-way ANOVA with Bonferroni post-test. (C is abbreviated from caspofungin, F is abbreviated from fluconazole)

Discussion

A limited number of *in vitro* studies thus far have evaluated the effects of sequential therapy of CAS followed by triazole against mixed *Candida spp*. biofilm. To our knowledge, this is the first study in which the efficacy of serial of sequential therapy of CAS and FLU against mixed *C. albicans* and *C. glabrata* biofilm was investigated after a short exposure to CAS or FLU. The objective of our study was to evaluate the impacts of sequential CAS/FLU treatment on biomass of biofilms. Numerous techniques were employed for the measurement of the quantity of cells in biofilms. These procedures vary widely as to their time and cost requirements. CV staining is a simple but reliable method for total biomass quantification because it binds to any negatively charged molecules within the cells and also the extracellular polymeric matrix. The amount of dye solubilized by the solvent is directly proportional to biofilm size. However, some drawbacks of CV were identified, such as its low reproducibility to give repeatable results and its inability to distinguish between live and dead cells.[15] The experimental condition of biofilm growth, the specific nature and concentration of the solvent and the elution time are crucial steps for CV assay. These issues may contribute to some large variations among the percentages of biofilm reduction of FLU- pretreated biofilms presented here. As CV staining does not allow us to discriminate between viable and nonviable cells, and our previous study demonstrated that simultaneous CAS/FLU combinations affected the cell architecture of mixed *C. albicans* and *C. glabrata* biofilm.[11] Therefore, the cell viability and microstructure of mixed *C. albicans* and *C. glabrata* biofilm in response to CAS/FLU sequential combination should

be further evaluated using some other techniques such as XTT assay, scanning electron microscope, or direct visualization using Confocal Scanning Laser Microscopy (CLSM) coupled with vital staining confocal. Furthermore, study more than one strain of *Candida* from a given species would be necessary to characterize the species-specific properties.

Our study demonstrated that the susceptibilities to CAS/FLU combinations of mixed *C. albicans* and *C. glabrata* biofilm pre-exposed to CAS for 1 - 6 hours, at 1 MIC (0.56 µg/ml) concentration, were identical. Similar results were revealed when mixed biofilms were treated with a range of subinhibitory concentration of CAS/FLU. An equivalent efficacy to reduce CAS-pretreated mixed biofilm at 0.25 to 0.5 MIC concentration (0.14 - 0.28 µg/ml) was exhibited compared to that of CAS treatment alone at 1 MIC. These results were similar to previous study in planktonic *C. albicans* cells exposed to CAS at the concentrations of 0.2 and 0.4 µg/ml for 4 hours followed by FLU at the concentrations ranged from 0.008- 4.0 µg/ml, which reported that treatment *C. albicans* cell with CAS followed by FLU was as effective as CAS treatment given alone.[16] Our results were also consistent with *in vitro* study of sequential therapy with CAS followed by posaconazole, a new triazole drug, of *C. glabrata* cells, which demonstrated that the susceptibilities to posaconazole of *C. glabrata* cells pre-exposed to CAS were identical to those of untreated cells.[17] These results may suggest that there is no therapeutic advantage in using the sequential therapy CAS followed by FLU, at least with the concentrations investigated here.

In contrast to what we observe with CAS followed by FLU regimen, the inhibitory effects of CAS/FLU combinations on FLU-pretreated mixed biofilm were dependent on the length of preincubation time of FLU. The longer period of pre-exposed to FLU, the lesser of antifungal activity of CAS/FLU combination on mixed biofilm. The significant decreasing of biomass inhibition effects of CAS/FLU combination on FLU-pretreated mixed biofilm at 4 - 6 hrs were demonstrated here. These results were in accordance with previous study revealed a significant decrease in the efficacy of CAS when *C. albicans* biofilm was firstly treated with FLU for 24 hours followed by another 24 hours of CAS treatment using XTT assay to investigate the viability of biofilm.[14] The fungistatic nature of the azoles towards *C. albicans* induces a strong directional selection on the surviving population to evolve drug resistance. The induced resistance to combinations subsequent to FLU treatment might relate to the induction of cellular stress response. Previous studies revealed that stress adaptation was crucial for *C. albicans* virulence as it increased the survival of this pathogen.[18, 19] Treatment of the biofilm with FLU first would result in the disruption of the cell membrane leading to osmotic stress. Candida biofilm might respond by producing an extensive polysaccharide and protein rich exopolymeric matrix for survival, as well as by regrowth of the biofilm. Stress adaptation then stabilizes the cell in the presence of drug and allows it to develop more profound resistance mechanisms over time. The mechanism of induced CAS resistance subsequent to FLU treatment was related to the induction of cellular stress response mediated by heat shock protein (Hsp) 90.[14] The similar result was also revealed with other echinocandin-triazole combination against *C. albicans* biofilms. Voriconazole significantly antagonized the fungicidal effect of micafungin against *C. albicans* biofilms, and Hsp90-related stress responses were involved in the antagonism.[20] Studies demonstrated that Hsp90 potentiated the crucial responses to cell wall stress exerted by the echinocandins by enabling the function of its client protein calcineurin, which allowed the fungus to survive otherwise lethal conditions.[21, 22]

Interestingly, the percentage of mixed biofilm reduction of CAS/FLU combinations was significantly decreased when mixed biofilm exposed to FLU at the concentration of 1 MIC (309 µg/ml) for 3 hours followed by CAS at the concentration of 1 MIC (0.56 µg/ml), compared to that of CAS monotherapy at 1 MIC. However, the efficacy of CAS/FLU combinations to reduce biofilm was indifferent to that of CAS monotherapy at 1 MIC when mixed biofilm exposed to FLU at the concentration of 0.5 MIC (154 µg/ml) for 3 hours followed by CAS at the concentration of 0.5 MIC (0.28 µg/ml). These results were in agreement with previous report by Sarkar,[14] which found that *C. albicans* biofilms pretreated with higher concentration of FLU demonstrated higher resistance to CAS. In contrast to what we found form the 3 hrs FLU-pretreated mixed biofilm, the inhibition effects of CAS/FLU combinations against 4 hrs FLU-pretreated mixed biofilm at every concentration were significantly decreased, compared to those of single drug treatment. These results may indicate that this diminished activity of CAS/FLU on FLU-pretreated mixed biofilm dependent on the concentration of FLU and CAS used and the duration of pre-exposure time. The combination of CAS at half MIC (0.28 µg/ml) with FLU at half MIC (154.5 µg/ml) exhibiting an equivalent efficacy to reduce the 3 hrs FLU-pretreated biofilm compare to CAS alone at 1 MIC concentration observed here may have implication for clinical management of patients with candidiasis. However, results of *in vitro* studies cannot yet be translated into clinical decisions because of the different methodology and the complexity of clinical scenarios.

In conclusion, our data revealed that both sequence of drugs and the length of pre-exposure time affected the inhibitory activity of CAS/FLU combinations on the biomass of mixed *Candida albicans* and *Candida glabrata* biofilm. As in the case of CAS-FLU sequential therapy, treatment of mixed *C. albican* and *C. glabrata* biofilm by fluconazole, followed by another 24 hour of CAS treatment, a significant decrease in the efficacy of CAS/FLU combinations was demonstrated. However, these observations were not found in CAS-pretreated mixed biofilm.

References

1. Marukutira T, Huprikar S, Azie N, Quan SP, Meier-Kriesche HU, Horn DL. Clinical characteristics and outcomes in 303 HIV-infected patients with invasive fungal infections: data from the prospective antifungal therapy alliance registry, a multicenter, observational study. HIV AIDS (Auckl) 2014;6:39-47.
2. Kauffman C. Fungal infections in older adults. Clin Infect Dis 2001;33:550-5.
3. Horn DL, Neofytos D, Anaissie EJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin Infect Dis 2009; 48:1695-1703.
4. Muadcheingka T, Tantivitayakul P. Distribution of *Candida albicans* and non-*albicans Candida* species in oral candidiasis patients: correlation between cell surface hydrophobicity and biofilm forming activities. Arch Oral Biol 2015;60:894-901.
5. Pappas PG, Kauffman CA, Andes DR, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 2016;62:e1-e50.
6. Taff HT, Mitchell KF, Edward JA, Andres DR. Mechanisms of Candida biofilm drug resistance. Future Microbiol 2013;8:1325-37.
7. Flevari A, Theodorakopoulou M, Velegraki A, Armaganidis A, Dimopoulos G. Treatment of invasive candidiasis in the elderly: a review. Clin Intervent Aging 2013;8:1199-1208.

8. Chandra J, Mukherjee PK, Leidich SD, et al. Antifungal resistance of candidal biofilms formed on denture acrylic *in vitro*. J Dent Res 2001;80:903-8.
9. Ramage G, Wickes BL, Lopez-Ribot JL. Biofilms of *Candida albicans* and their associated resistance to antifungal agents. Am Clin Lab 2001;20:42-4.
10. Mukherjee PK, Chandra J. Candida biofilm resistance. Drug Resist Update 2004;7:301-9.
11. Pesee S, Angkananuwat C, Tancharoensukjit S, et al. *In vitro* activity of caspofungin combined with fluconazole on mixed *Candida albicans* and *Candida glabrata* biofilm. Med Mycol 2016;54:384-93.
12. Tobudic S, Kratzer C, Lassnigg A, Presterl E. Antifungal susceptibility of *Candida albicans* in biofilms. Mycoses 2012;55:199-204.
13. Kontoyiannis DP, Lewis RE. Toward more effective antifungal therapy: the prospects of combination therapy. Br J Heamatol 2004;126:165-75.
14. Sarkar S, Uppuluri P, Pierce CG, Lopez-Ribot JL. *In vitro* study of sequential fluconazole and caspofungin treatment against *Candida albicans* biofilms. Antimicrob Agents Chemother 2014;58:1183-6.
15. Peeters E, Nelis H, Coenye T. Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. J Microbiol Method 2008;72:157-65.
16. Barchiesi F, Spreghini E, Baldassarri I, et al. Sequential therapy with caspofungin and fluconazole for *Candida albicans* infection. Antimicrob Agents Chemother 2004;48:4056-8.
17. Spreghini E, Maida CM, Milici ME, Scalise G, Barchiesi F. Posaconazole activity against *Candida glabrata* after exposure to caspofungin or amphotericin B. Antimicrob Agents Chemother 2008;52:513-7.
18. Arana D, Alonso-Monge R, Du C, Calderone R, Pla J. Differential susceptibility of mitogen-activated protein kinase pathway mutants to oxidative-mediated killing by phagocytes in the fungal pathogen *Candida albicans*. Cell Microbiol 2007;9:1647-59.
19. Patterson M, McKenzie C, Smith D, et al. Ybp1 and Gpx3 signaling in *Candida albicans* govern hydrogen peroxidase-induced oxidation of the Cap1 transcription factor and macrophage escape. Antioxid Redox Signal 2013;19:2244-60.
20. Kaneko Y, Ohno H, Fukazawa H, et al. Anti-Candida biofilm activity of micafungin is attenuated by voriconazole but restored by pharmacological inhibition of Hsp90-related stress responses. Med Mycol 2010;48:606-12.
21. Singh S, Robbins N, Zaas A, Schell W, Perfect J, Cowen L. Hsp90 governs echinocandin resistance in the pathogenic yeast *Candida albicans* via calcineurin. PLoS Pathog 2009; 5:e10000532.
22. Cowen L, Lindquist S. Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. Sci 2005;309:2185-9.

บทคัดย่อ

ผลของการใช้ยาผสมระหว่างแคสโปฟังจินและฟลูโคนาโซลแบบตามลำดับต่อมวลชีวภาพของแผ่นชีวภาพผสมของเชื้อราแคนดิดา อัลบิแคนส์และแคนดิดา กลาบราต้าในห้องปฏิบัติการ

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บทนำ: การศึกษาที่ผ่านมาพบว่าประสิทธิผลของยาแคสโปฟังจินจะลดลงอย่างมีนัยสำคัญหากแผ่นชีวภาพของเชื้อราแคนดิดา อัลบิแคนส์ได้รับยาฟลูโคนาโซลมาก่อน ดังนั้น การศึกษานี้จึงมีจุดประสงค์เพื่อทดสอบประสิทธิผลของการผสมยาแคสโปฟังจินและฟลูโคนาโซลแบบตามลำดับต่อการกำจัดแผ่นชีวภาพผสมของเชื้อราแคนดิดา อัลบิแคนส์และแคนดิดา กลาบราต้าในห้องปฏิบัติการ

วิธีการศึกษา: แผ่นชีวภาพผสมของเชื้อราแคนดิดา อัลบิแคนส์และแคนดิดา กลาบราต้าจะได้รับยาตัวแรกที่ความเข้มข้น 1 MIC (แคสโปฟังจิน หรือฟลูโคนาโซล) ก่อนเป็นเวลา 1 ถึง 6 ชั่วโมง หลังจากนั้น ยาตัวที่สองที่ความเข้มข้น 1 MIC (ฟลูโคนาโซล หรือ แคสโปฟังจิน) จะถูกใส่เพิ่มเพื่อเป็นยาผสม ปริมาณแผ่นชีวภาพที่เหลืออยู่หลังจากแผ่นชีวภาพได้รับยาผสมจะถูกวัดโดยการย้อมสีคริสตัลไวโอเลต นอกจากนี้ การศึกษานี้ยังได้ทดสอบประสิทธิผลของยาผสมแบบตามลำดับเมื่อผสมในความเข้มข้นที่ต่างกัน โดยทดสอบบนแผ่นชีวภาพที่ได้รับยาตัวแรกก่อนที่เวลา 3 หรือ 4 ชั่วโมง

ผลการศึกษา: แผ่นชีวภาพผสมซึ่งได้รับยาแคสโปฟังจินก่อนในช่วงเวลาที่ต่างกัน จะไม่พบความแตกต่างของประสิทธิผลของยาผสมแคสโปฟังจินและฟลูโคนาโซลต่อปริมาณของแผ่นชีวภาพ อย่างไรก็ตาม ประสิทธิผลของยาผสมต่อปริมาณแผ่นชีวภาพผสมซึ่งได้รับยาฟลูโคนาโซลก่อนจะลดลงอย่างมีนัยสำคัญเมื่อแผ่นชีวภาพได้รับยาฟลูโคนาโซลก่อนในเวลาที่นานขึ้น *(P<0.001)* สำหรับแผ่นชีวภาพผสมที่ได้รับยาฟลูโคนาโซลก่อนเป็นเวลา 3 ชั่วโมง การได้รับยาแคสโปฟังจินร่วมกับฟลูโคนาโซลที่ความเข้มข้น 0.5 เท่าของค่า MIC จะมีผลทำให้ ร้อยละการลดลงของแผ่นชีวภาพเพิ่มขึ้นอย่างมีนัยสำคัญ (ร้อยละ 39.17) เมื่อเปรียบเทียบกับกลุ่มที่ให้ยาผสมที่ความเข้มข้น 1 เท่าของค่า MIC (ร้อยละ 12.52) *(P<0.05)*

สรุปผลการศึกษา: ประสิทธิผลของยาผสมแคสโปฟังจินและฟลูโคนาโซลต่อมวลชีวภาพของแผ่นชีวภาพผสมของเชื้อราแคนดิดา อัลบิแคนส์และแคนดิดา กลาบราต้าขึ้นอยู่กับลำดับการให้ยาและช่วงระยะเวลาที่ได้รับยาตัวแรก อย่างไรก็ตาม ผลของยาผสมแคสโปฟังจินและฟลูโคนาโซลแบบให้ตามลำดับต่อความมีชีวิตและโครงสร้างจุลภาคของแผ่นชีวภาพผสมของเชื้อราแคนดิดา อัลบิแคนส์และแคนดิดา กลาบราต้า ควรได้รับการศึกษาต่อไป

คำสำคัญ: แผ่นชีวภาพ, แคนดิดา อัลบิแคนส์, แคนดิดา กลาบราต้า, แคสโปฟังจิน, ฟลูโคนาโซล